SPATIO-TEMPORAL ECOLOGY AND BIOLOGY OF *LISTRONOTUS MACULICOLLIS* KIRBY (COLEOPTERA: CURCULIONIDAE) IN NEW YORK GOLF GOURSES

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SPATIO-TEMPORAL ECOLOGY AND BIOLOGY OF *LISTRONOTUS MACULICOLLIS* KIRBY (COLEOPTERA: CURCULIONIDAE) IN NEW YORK GOLF GOURSES

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Listronotus maculicollis Kirby (Coleoptera: Curculionidae) is a serious pest of golf course turf in the northeastern United States and eastern Canadian provinces. To enhance current management strategies and to introduce novel management approaches, phenological, ecological and physiological characteristics of *L. maculicollis* were investigated.

A three-parameter nonlinear logistic model, using 7.2 ˚C as a lower developmental threshold, was fitted to field-collected data of different developmental stages of two beetle populations from golf courses in New York and described their population accumulation patterns as a function of the degree-day accumulation within ± 7 days. The best models were further tested in a validation study encompassing sites that represented geographic/climatic ranges where *L. maculicollis* was known as a pest. Observed data tended to follow model predictions with respect to general pattern. Discrepancies were primarily due to faster population accumulation rates of each stage and/or greater synchrony than predicted. The results indicated site-specific variations in phenology. Further investigation about the effects of phenotypic plasticity, photoperiod, and adaptation to local habitat conditions on the phenology was suggested.

The spatio-temporal dynamics *of L. maculicollis*, *P. annua*, and damage were investigated. Damage aggregated at the periphery of the fairway, the primary reproduction site

for the beetle. It was spatially associated with larval density, thatch depth, and soil nitrate concentration. The crown feeding larvae and thatch (surface organic matter) were significantly associated with the occurrence of the damage. Association analysis indicated the crown feeding larvae were the primary cause of the damage and thatch provided a physical environment that was suitable for insect feeding and/or antagonistic to the plant's tolerance to the feeding. There was no significant association between *P. annua* density and plant damage or insect density. This result indicated that *L. maculicollis* pattern of colonization of turf areas was due to the physical environment that transitioned from high-mown turf to short-mown turf rather than a particular preference for *P. annua*.

The fecundity of field-collected adults greatly differed between spring and summer. After the immigration to fairways from the overwintering site in April, the overwintered adults continuously oviposited eggs through June with great individual variation in number (0-84 eggs/individual). On the other hand, in July, when the majority of the field-collected adults were considered to be new adults of the following generation, they rarely laid eggs. The results challenge the current interpretation of *L. maculicollis* voltinism and indicated that the appearance of multiple generations might arise from the variation in the number of eggs oviposited by the overwintered adults and the continuous immigration of overwintered adults. Rearing methods with artificial diet and plant material were investigated. The developmental times of the insect, from egg to adult, on lettuce diet were normally distributed with large variance (25-113 days). This variation is a possible explanation of observed asynchronous populations in the field. The results imply the necessity of modifying the conventional views of the seasonal changes of *L. maculicollis* development that were largely based on anecdotal observation.

BIOGRAPHICAL SKETCH

Masanori Seto was born on Janauary 14, 1980 in Tokyo, Japan. He was raised in Tachikawa city and graduated TOHO Junior and Senior High School in 1998. He entered Tokyo University of Agriculture and Technology (TUAT) in 1999 and completed the undergraduate program for five semesters. Prior to receiving a Bachelor of Agricultural Science (Environmental and Natural Resource Science) in 2003, he attended Purdue University in West Lafayette, Indiana for 16 months as an exchange student funded by Japanese government. Upon his return to Japan, Masanori enrolled as a graduate student in the Department of Environmental Science on Biosphere at TUAT. He obtained his Master of Agricultural Science in 2005, focusing on microbial denitrification process in a tertiary wastewater treatment system by use of phosphorus sorbent and porous concrete under the guidance of Drs. Masayuki Seto, Yoko Katayama, and Mitsunori Tarao. In 2006, he joined as a member of Soil Insect Ecology & Turfgrass Entomology Lab in the Department of Entomology at Cornell University/New York State Agricultural Experiment Station in Geneva, New York in pursuit of his Ph.D. His doctoral research focused on spatial ecology and biology of *Listronotus maculicollis* in New York golf courses. After graduation, he will pursue a research career in agriculture; become a farmer who works in the fields on fine days and drumming Taiko on rainy days.

This dissertation is dedicated to my grandfather, Masayoshi Seto.

本論文を祖父 瀬戸昌義に捧ぐ。

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CHAPTER ONE

NONLINEAR DEGREE-DAY MODEL TO PREDICT PHENOLOGY OF *LISTRONOTUS MACULICOLLIS* KIRBY (COLEOPTERA: CURCULIONIDAE) IN GOLF COURSE LANDSCAPES

ABSTRACT

Listronotus maculicollis Kirby (Coleoptera: Curculionidae) causes severe damage to *Poa annua* turf in the Northeast U.S. Its spring emergence roughly correlates with bloom of *Forsythia* spp., but the relationship between *L. maculicollis* development and temperature is poorly understood. Simple predictive models, such as degree-day models, may be a useful tool for improving management of *L. maculicollis,* which currently relies on chemical controls targeting overwintered adults and larvae of the first generation to prevent damage. In this study, the relationship between field collected population data of different developmental stages of *L. maculicollis* and degree-days (DD) was developed into a predictive model. Using data collected from two golf courses in Upstate New York, we evaluated a nonlinear logistic model for predicting the timing of *L. maculicollis* population using five different lower developmental threshold (base) estimates. The logistic model closely described the relationship between insect population accumulation and accumulated DD for all developmental stages, while the most useful base temperature differed among developmental stages. The model for overwintered adults using base 0 °C predicted 10% emergence at 258 DD, 50% at 690 DD, and 90% at 1153 DD. The model using a base 7.2 °C predicted 10% emergence of the first generation larvae at 114 DD, 50% at 304 DD, and 90% at 485 DD. The potential of using degree-day models for timing scouting efforts and pesticide applications is discussed in relation to these results.

INTRODUCTION

Because poikilothermal development is directly related to temperature and time (Allen 1976, Bonhomme 2000), degree-day (DD) models are practical tools in ecology and pest management to describe the development and phenology of insects in the field. As the most common expression of heat accumulation, DD is estimated by measuring the area contained under the trace of a thermograph chart but above a lower developmental threshold temperature (base). The estimation of heat units by measuring the area under a sine curve, as proposed by Arnold (1960), is a simple technique that requires only maximum and minimum daily temperatures (Baskerville et al. 1969). Due to the ease of use, DD models can be practical, interpretive and decision-making tools for pest management practitioners in diverse management systems (Agnello et al. 1993, Kulhanek 2009).

Golf course management in the Northeastern United States (U.S.) is increasingly challenged by *Listronotus maculicollis* Kirby (Coleoptera: Curculionidae), a destructive insect pest of annual bluegrass, *Poa annua* L. (McGraw and Koppenhöfer 2008). Larvae cause damage to short-mown annual bluegrass turf, such as fairways, greens and tees. Protecting playing surfaces from *L. maculicollis* is of great concern to golf course superintendents since the damage severely degrades aesthetic and playing quality. Once an issue largely limited to the Northeastern U.S., the range of problematic infestations is expanding to the southern Mid-Atlantic, Midwest, and Southern Canada (Vittum 1999, Simard et al. 2007, Diaz & Peck 2007, Koenig et al. 2008). Diaz and Peck (2007) reported that *L. maculicollis* overwinter as adults in various microenvironments up to 60 m away from golf course playing surfaces and largely along the edge of defined tree lines. Overwintered adults immigrate to golf courses for development and reproduction in early spring. The first generation completes its life cycle by late spring to early

summer and visual damage on infested turf appears concurrently. Late-stage larvae (instars 4 and 5) of the first generation are considered the major damaging agents (Vittum et al. 1999). The two targets for prevention of the damage are the immigrating overwintered adults prior to the initiation of oviposition, and the larvae of the first generation before the appearance of damage.

Golf course superintendents have traditionally relied on plant phenological cues to target immigrating overwintered adults (Tashiro & Straub 1973, Vittum et al. 1999). This phenological strategy entails an insecticide application sometime between full bloom of *Forsythia* spp. and full bract of *Cornus florida* L., which yields a relatively wide window of timing (ca. 14 – 28 days) for control. Such a wide control window encourages multiple insecticide applications against a single generation. Multiple applications and the overwhelming historical reliance on pyrethroid insecticides have led to the development of insecticide resistance among certain populations of *L. maculicollis* (Ramoutar et al. 2009). To overcome these control challenges, it would be helpful to develop a model that can more precisely predict *L. maculicollis* population flux on golf course landscapes over time and is applicable for *L. maculicollis* population across wide geographical areas and different years. Thus, the model needs to be developed in relation to variables that mitigate yearly and geographical variations. As temperature is a universal variable that influences insect development, a temperature-dependent population model should be explored as a new management tool.

Two studies have addressed the relationship between *L. maculicollis* development and temperature. Vittum (1980) showed that the temporal occurrence of first generation larvae was better predicted based on Julian date (JD) than DD, but second generation larvae were better predicted with DD. In that study, the base temperature was set at 11.1 °C according to the lower developmental temperature threshold for pupal development derived from laboratory

observations. In the second study, Rothwell (2003), working with instars 3-5, determined 13.3 °C to be an appropriate base temperature for larval growth in a laboratory experiment. A subsequent three-year field survey determined larvae occurred in the field around 150-200 $DD_{13.3}$ °C. Despite their ecological and economic importance, the relationship between the emergence and peak occurrence of overwintered *L. maculicollis* adults and temperature was not investigated in either study. Neither was the emergence and development of each larval stage examined in relation to temperature accumulation.

Understanding *L. maculicollis* phenology in the context of temperature may enhance management practices by enabling more efficient scouting and better-timed insecticide applications. Nonlinear logistic DD models may have the potential to complement or supersede the current reliance on phenological plant predictors**.** The primary objective of this study, therefore, was to develop DD models for predicting the timing of *L. maculicollis* overwintered adult emergence and the peak occurrence of developmental stages based on a phenological analysis of *L. maculicollis* population data reported by Diaz et al. (2008).

MATERIALS AND METHODS

Study sites and data collection

Details of the site description, collection methods, and identification of developmental stages were presented in Diaz et al. (2008). Briefly, populations of *L. maculicollis* were monitored weekly on a fairway at each of two golf courses in Central New York: Ithaca (Robert Trent Jones Golf Course at Cornell University) and Fayetteville (Onondaga Golf & Country Club). Field collections were made from spring through fall over three consecutive seasons (2004-2006). Three transects were laid out perpendicular to the length of each fairway. In each

transect, there were 5 sampling points on the fairway and 2 on the intermediate rough. At each sampling point, adults were collected from a 0.25-m^2 area demarcated by a circular template placed on the ground. After mixing 15 ml of liquid dish detergent in 4 liters of water, we poured this solution over the area within each template. After 2-5 min, irritated by the soap solution, adults ascended to the top of the leaf blade where we collected them with an aspirator. In addition, four 6.0-cm diameter samples were removed at each sampling point with a turf-corer (TTP1-M Turf-Tec Tubular Turf Plugger, Turf-Tec International, Tallahassee, FL) to a depth of 6.4 cm. In the lab, each core was placed in a 133-ml plastic cup with a screened bottom (aluminum screen, 7.6 x 7.6 mm mesh size) held inside a 163-ml cup containing ≈5 ml of glycerin to capture the larvae. Cups were placed in a controlled climate chamber for 3-4 d at 30ºC, which forced larvae out of the core, through the screen, and down into the glycerin. This method allowed the extraction of all stages of larvae (instar 1 through 5), but not the immobile pupae. All collected adults were identified to gender (male or female) and maturation (callow or mature), and all larvae were identified to instar. A callow adult is a newly emerged adult whose integument is soft and incompletely sclerotized (light brown in contrast to black of mature adults).

Population fluctuation curves, which describe an increase or decrease of insect density over time, were generated for collected data of developmental stages for each site and each year (Diaz et al. 2008). Demarcation of the distinct generations was based on the pattern of the population fluctuation curve (i.e., rise and fall) and the emergence of callow adults as an indicator of the commencement of the subsequent generation. The developmental stages from each generation, site and year were modeled in this study. Adult male and female data were pooled because they exhibited almost indistinguishable population fluctuation curves for each

site by year (Diaz et al. 2008). Early-stage larvae of *L. maculicollis* feed within the host plant as stem borers while late-stage larvae live at the soil surface to feed on the crown. Therefore, instars 1, 2 and 3 were also pooled as the stem boring stage and instars 3, 4, and 5 as the crown feeding stage, which is considered the principal source of economic damage. Instar 3 was counted in both groups as it is assumed to be a transitional stage.

Model construction and selection

Daily maximum and minimum temperatures were obtained from on-site weather stations at each golf course. On the few occasions when data were missing, gaps were filled with data from the nearest National Weather Service Station. DD were estimated as follows (Arnold, 1960):

$$DD = \sum \left[\frac{(T_{max} + T_{min})}{2} - (T_{base}) \right]_n$$

where T_{max} is the daily maximum temperature, T_{min} is the daily minimum temperature, T_{base} is the lower developmental threshold temperature below which growth processes cease, and n is the day number. Negative DD were assigned values of zero.

DD accumulation was calculated from three different start dates: January 1, February 1, and March 1. We evaluated five different lower developmental base temperatures: 0, 4.4, 7.2, 10.0, and 13.3 °C. Base 0 °C was included because a base temperature below which development ceases may be more accurate and have a better precision error rate (Mailloux and Bostanian 1993). Base 4.4 °C was included because it was the minimum temperature at which field collected adults were observed to stay active in the laboratory setting (MS, personal observation). Base 10.0 °C was included as it is a lower developmental threshold used for the degree-day model of the rice water weevil, *Lissorhoptrus oryzophilus* Kuschel (Zou etal. 2004).

Base 7.2 °C was included as a median threshold between base 4.4°C and 10.0°C. Base 13.3°C was included based on a laboratory study on *L. maculicollis* larvae that showed that larval growth was most consistently described by the model using a base of 13.3°C (Rothwell 2003).

We hypothesized that the relationship between DD and individuals in a particular developmental stage was nonlinear logistic with an approximately bell-shaped curve (insect counts increase with DD accumulation to a peak and then decrease as the season progresses) based on the population fluctuation curve presented by Diaz et al. (2008). Count data of each developmental stage were transformed into cumulative fractions and were analyzed as a function relating the cumulative fraction of counts to accumulated DD. Regression analysis and graphing were conducted using R, version 2.14.0 (R Development Core Team 2011) and SigmaPlot (Systat Software Inc. 2010). The nonlinear logistic function was as follows:

$$P = \frac{\alpha}{1 + e^{(\frac{\beta - DD}{\gamma})}}$$

where P is the cumulative fraction of *L. maculicollis* developmental stage, α is the horizontal asymptote (expected to be approximately 1), β is the value of DD at the inflection point, and γ determines the steepness at inflection (the lower γ gives the steeper slope). Akaike's Information Criterion (AIC) (Akaike 1981) was used for model selection. AIC requires a bias-adjustment for small sample sizes, when the ratio of n/K < 40 (Cavanaugh 1997). Therefore, AIC was calculated as follows:

$$AIC = n * \ln(RSS/n) + 2 * K + (2 * K * (K+1))/(n - K - 1)$$

where RSS is the residual sums of squares, n is the number of observations and K is the number of parameters in the model. The model with the lowest AIC for each developmental stage was chosen as the best model and its P was plotted against DD. ΔAICs , AIC difference between the

best model and every other model, were also calculated to evaluate the relative significance of the different start dates for calculating DD accumulation and of different base temperatures.

RESULTS

Models for a particular combination of developmental stage and base temperature had identical or close to identical AIC values ($\Delta AIC < 1$) regardless of the different start dates for calculating DD accumulation. Thus, variation in start date had no effect on the model construction. For the sake of convenience, January 1 was set as the standard start date for the rest of the results section.

For adults, the model for overwintered adults had a better fit than those for the first and second generation adults based on R^2 values (Table 1). For larvae, the model for the first generation (all instars pooled) had a better fit than that for the second generation.

Emergence of the overwintered adults was best described using a fitted nonlinear logistic model with base temperature 0 °C ($F = 460.23$; $df = 2, 63$; p-value < 0.0001). The model predicted 10% emergence at 258 DD_0, 30% at 523 DD_0, 50% at 690 DD_0, 70% at 861 DD_0, and 90% at 1153 DD_0 (Fig. 1). ΔAIC was largest for the model with base temperature 13.3 °C ($\Delta AIC=58.65$). Adult emergence of the first generation was best described using a fitted logistic model with base temperature 7.2 °C ($F = 49.28$; $df = 2, 40$; p-value < 0.0001). ΔAIC was largest for the model with base temperature 13.3 °C ($\Delta AIC = 7.03$). Adult emergence of the second generation was best described using a fitted logistic model with base temperature 7.2 °C ($F = 25.48$; $df = 2, 20$; p-value < 0.0001). ΔAIC was largest for the model with base temperature 13.3 °C ($\Delta AIC = 7.00$).

For larvae (all instars pooled), emergence of the first generation was best described using

a fitted logistic model with base temperature 7.2 °C (F = 271.43; df = 2, 46; p-value < 0.0001). The model predicted 10% emergence at 114 $DD_{7.2}$, 30% at 231 $DD_{7.2}$, 50% at 304 $DD_{7.2}$, 70% at 377 $DD_{7.2}$, and 90% at 485 $DD_{7.2}$ (Fig. 2). ΔAIC was largest for the model with base temperature 13.3 °C (ΔAIC = 7.03). Larval emergence of the second generation was best described using a fitted logistic model with base temperature 7.2 °C (F = 67.18; df = 2, 45; p-value < 0.0001). ΔAIC was largest for the model with base temperature 13.3 °C (ΔAIC = 20.19).

For instar 1, emergence of the first generation was best described using a fitted logistic model with base temperature 0.0 °C (F = 198.36; df = 2, 46; p-value < 0.0001). ΔAIC was largest for the model with base temperature 13.3 °C (ΔAIC = 42.37). Emergence of the second generation was best described using a fitted logistic model with base temperature 7.2 °C (F = 22.59; df = 2, 33; p-value < 0.0001). ΔAIC was largest for the model with base temperature 13.3 °C (ΔAIC = 8.90).

For instar 2, emergence of the first generation was best described using a fitted logistic model with base temperature 7.2 °C (F = 159.47; df = 2, 40; p-value < 0.0001). ΔAIC was largest for the model with base temperature 13.3 °C (ΔAIC = 14.36). Emergence of the second generation was best described using a fitted logistic model with base temperature 7.2 °C (F = 53.62; df = 2, 28; p-value < 0.0001). ΔAIC was largest for the model with base temperature 13.3 °C (ΔAIC = 11.53).

For instar 3, emergence of the first generation was best described using a fitted logistic model with base temperature 7.2 °C (F = 162.51; df = 2, 39; p-value < 0.0001). ΔAIC was largest for the model with base temperature 13.3 °C (ΔAIC = 15.21). Emergence of the second generation was best described using a fitted logistic model with base temperature 13.3°C (F = 189.26; df = 2, 22; p-value < 0.0001). ΔAIC was largest for the model with base temperature 0.0

°C (ΔAIC = 24.73).

For instar 4, emergence of the first generation was best described using a fitted logistic model with base temperature 7.2 °C (F = 168.02; df = 2, 43; p-value < 0.0001). ΔAIC was largest for the model with base temperature 13.3 °C (ΔAIC = 16.45). Emergence of the second generation was best described using a fitted logistic model with base temperature 0.0°C (F = 34.79; df = 2, 27; p-value < 0.0001). ΔAIC was largest for the model with base temperature 13.3 °C (ΔAIC = 14.49).

For instar 5, emergence of the first generation was best described using a fitted logistic model with base temperature 7.2 °C (F = 285.07; df = 2, 46; p-value < 0.0001). ΔAIC was largest for the model with base temperature 13.3 °C (ΔAIC = 30.54). Emergence of the second generation was best described using a fitted logistic model with base temperature 0.0°C (F = 239.79; df = 2, 24; p-value < 0.0001). ΔAIC was largest for the model with base temperature 13.3 °C (ΔAIC = 26.29).

For the stem boring stage, emergence of the first generation was best described using a fitted logistic model with base temperature 7.2 °C (F = 356.39; df = 2, 46; p-value < 0.0001). The model predicted 10% emergence at 80 $DD_{7.2}$, 30% at 191 $DD_{7.2}$, 50% at 260 $DD_{7.2}$, 70% at 329 $DD_{7.2}$, and 90% at 438 $DD_{7.2}$ (Fig. 3). ΔAIC was largest for the model with base temperature 13.3 °C (ΔAIC = 38.37).

For the crown feeding stage, emergence of the first generation was best described using a fitted logistic model with base temperature 7.2 °C (F = 229.46; df = 2, 46; p-value < 0.0001). The model predicted 10% emergence at 184 $DD_{7.2}$, 30% at 290 $DD_{7.2}$, 50% at 357 $DD_{7.2}$, 70% at 423 $DD_{7.2}$, and 90% at 527 $DD_{7.2}$ (Fig. 4). ΔAIC was largest for the model with base temperature 13.3 °C (ΔAIC = 19.42).

The logistic models with base temperature 7.2 °C yielded the best predictors for most developmental stages. Even for the developmental stages whose best models did not employ base temperature 7.2 °C, using base temperature 7.2 °C made little difference (e.g. 8.23 in the first-generation instar 1) in ΔAICs compared to those of the best models (Table 1 and 2). For comparison, Table 2 lists the coefficients and predicted DD of the models with base temperature 7.2 °C for each developmental stage. For the second-generation instar 4, a limitation in the model ($a < 0.9$). precluded prediction of 90% emergence.

The equations for each developmental stage from Table 2 are graphed in Figure 5. The presence of overwintered adults overlapped that of first generation larvae as well as the early stage of first-generation adults. Moreover, the presence of first generation adults overlapped the entire period of second-generation larvae. In the first generation, larval instars showed a sequential manner of accumulation. Older larval stages developed faster than earlier stages as indicated by the increase of x_0 values in Table 2. In the second generation, on the other hand, the pattern of sequential accumulation was obscured. The population accumulation of instar 1 occurred slowly, i.e., a gentle slope, and the accumulation of instar 3 and 4 occurred concurrently (Table 2). Through the season, almost all developmental stages of *L. maculicollis* were found on any given day; in other words, the periods of occurrence of all developmental stages in each generation overlapped most of the time.

DISCUSSION

The correspondence between all developmental stages and DD was characterized by a nonlinear logistic function (Table 1). The cumulative emergence of overwintered adults was well explained by DD ($R^2 = 0.94$). The previous population surveys showed the immigration of adults

from overwintering sites to the reproductive sites and the population flux on the reproductive sites tended to exhibit bimodal or trimodal patterns with significant variations in abundance from year to year (Diaz and Peck. 2007, Diaz et al. 2008). Nevertheless, the nonlinear logistic model was robust enough to mitigate such variations and indicated that the cumulative emergence of overwintered adults on the reproductive sites followed a bell-shaped pattern in relation to DD. This implies that the migration and emergence of overwintered adults at both sites were similarly regulated by DD.

The cumulative occurrence of larval stages of the first generation was also well explained by DD (R^2= 0.89 - 0.93) (Table 1). The patterns of population fluctuations of larval stages of the first generation were influenced by the emergence patterns of overwintered adults (Diaz et al. 2008), however, the nonlinear logistic models showed that the patterns of cumulative occurrence followed a bell-shaped curve in relation to DD. Thus, the cumulative occurrence of larval stages of the first generation is predictable with common DD.

Base temperature 7.2 °C seems to be suitable to describe the population accumulation pattern of any developmental stage (Table 1 and 2). The difference in R^2 and AIC between the best model and the other models was minimal and was considered not to seriously impact the practicality of the models, except for the models with base temperature 13.3 °C. With the exception of second generation instar 3, the models with base temperature 13.3 °C had the greatest ΔAIC among each group of models. This result was contrary to Rothwell's result (2003), which suggested 13.3 °C as the best base temperature based on laboratory rearing of instar 3-5. Until theoretical developmental thresholds for each developmental stage are determined under laboratory conditions, which will be possible once practical rearing methods are developed, we propose that base temperature of 7.2 °C be used as a standard.

The r-squared values of the models for developmental stages of the second generation (0.58 – 0.95) were more variable than those of the first (0.89 – 0.93) (Table 1). There are several possible explanations. The two generations were subjectively demarcated based on the emergence of callow adults and the shape of the population fluctuation curves. There was no absolute break in the population fluctuation curves between generations in all developmental stages. Thus, we inevitably counted overwintered adults as a part of the first generation, and overwintered adults and first generation adults as a part of the second generation. When the longevity of overwintered adults was followed under laboratory conditions, they survived more than 2 yr and retained the capacity to reproduce for at least 1 yr (MS, personal observation). These observations, along with the modeling results, support the idea of significant overlap between generations. In the same context, it is unclear which adults (overwintered or first generation) produce eggs after the emergence of the first generation callow adults. Assuming the first generation adult is reproductively mature upon emergence, there would be continuous oviposition by the overwintered adults and the first generation adult, and the distinction between the preceding and following populations might be lost. The inability to clearly distinguish two generations might have contributed to the decrease in model R^2 values between instar 1 of the second ($R^2 = 0.58$) and first ($R^2 = 0.91$) generations (Table 1). Due to overlapping generations, the prediction of this developmental stage was disrupted after the emergence of the first generation.

The long-term immigration and continuous oviposition of overwintered adults also contributed to overlapping generations, in which all or most developmental stages were present at any time (Table 2 & Fig. 5). This contributed to the difficulty in identifying population accumulation patterns for certain generations of the insect. Nevertheless, the models describing

phenology of the overwintered adults, and larvae of the first generation (pooled, stem boring stage, crown feeding), all explained a high degree of the variation R^2: 0.91 – 0.94 (Figs. 1-4). Therefore the models have potential to predict the emergence pattern of these stages, which are the primary targets for insecticidal control. Field tests, however, will be necessary to determine which temperature-driven model (overwintered adults, first generation larvae, stem boring, or crown feeding) is the most effective in predicting the timing of management activities reducing visual damage to golf courses. There is currently no information available for the optimum timing of control based on DD. To suppress the visual damage of *L. maculicollis*, it would be realistic to explore spray timing based on DD with evaluation of control and visual damage.

The relationship between temperature and development has been documented for a related pest, the carrot weevil, *Listronotus oregonensis* L. (Simonet and Davenport 1981). The temperature thresholds and thermal requirements for development and reproduction were determined for this insect in a laboratory study. A DD model was developed based on these laboratory estimates for all the different developmental stages using base temperature 7.0 °C. A field study was in good agreement with the laboratory data showing that the newly emerged first generation adults occurred at 139 DD. The authors concluded that combining results from both the laboratory and field were very useful in timing sampling intervals and spray applications and most importantly in following population development. The predictability of the phenology of *L. oregonensis*, which has a life history similar to *L. maculicollis*, substantiates the applicability of the models developed in the current paper.

Golf courses located in southern NY or NJ experience the emergence of overwintered adults much earlier than our experimental sites (McGraw and Koppenhöfer 2009). Future work should therefore aim to confirm the effectiveness of our models, which were based on population

data collected in two golf courses in central NY over three years that we only 70 km apart and within USDA plant hardiness zone 5a. Significant injury from *L. maculicollis*, once limited to the Northeast, is expanding to the southern Mid-Atlantic, Midwest, and Southern Canada (Vittum 1999, Simard et al. 2007, Diaz & Peck 2007, Koenig et al. 2008), ranging from 3b to 7a of USDA plant hardiness zone. The models need to be validated across these geographical areas. In addition, model precision may be improved through laboratory studies to refine estimates of temperature thresholds and thermal requirements for development and reproduction of *L. maculicollis*.

Nonlinear logistic DD models suitably described the emergence and development of *L. maculicollis* life stages that are economically important (overwintered adults and the first generation larvae.). The models will be most useful when they are evaluated in conjunction with various control measures. As new intervention tactics (insecticidal, biological or cultural) are developed, this model may allow us to target potentially vulnerable developmental stages in time to minimize damage.

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Table 1. The best model selected for each developmental stage. Nonlinear model $P= \alpha/(1+\exp((\beta-DD)/\lambda)$ where P is cumulative fraction emergence of each life stage, α, β, and γ are model coefficients, and DD is the independent variable (degree days).

Generation	Developmental stage	Variable (DD)	R^2	AIC	α	β	γ
Overwintered	Adult	DD0	0.94	-318.56	0.98	682.94	194.87
First generation	All larvae	DD7.2	0.92	-220.72	1.10	307.92	87.62
	Instar 1	DD0	0.91	-179.94	0.98	700.65	94.73
	Instar 2	DD7.2	0.89	-179.23	1.02	280.78	85.06
	Instar 3	DD7.2	0.89	-173.57	1.05	315.87	83.13
	Instar 4	DD7.2	0.89	-182.26	1.01	349.63	68.01
	Instar 5	DD7.2	0.93	-206.76	0.98	423.54	50.25
	Adult	DD7.2	0.71	-151.79	0.97	812.83	140.03
Second generatio	All larvae	DD4.4	0.75	-166.92	1.01	1271.53	186.28
	Instar 1	DD7.2	0.58	-105.47	0.93	894.89	154.27
	Instar 2	DD7.2	0.79	-107.87	0.95	919.88	102.50
	Instar 3	DD13.3	0.95	-113.66	0.98	499.74	60.52
	Instar 4	DD0	0.72	-95.10	0.92	1930.01	178.99
	Instar 5	DD0	0.95	-127.39	0.99	2090.08	170.06
	Adult	DD7.2	0.72	-78.49	1.00	1432.44	119.95

Table 2. The nonlinear logistic models with base temperature 7.2 for all developmental stages. Nonlinear model P= α/(1+exp((β-DD)/λ) where P is cumulative fraction emergence of each life stage, α, β, and γ are model coefficients, and DD is the independent variable (degree days).

Generation	Developmental stage	R^2	AIC	α	β	γ	% cumulative emergence				
							10%	30%	50%	70%	90%
Overwintered	Adult	0.92	-306.40	0.95	219.88	93.56	20	148	231	262	499
First generation	All larvae	0.92	-220.72	1.10	307.92	87.62	106	222	292	329	439
	Instar 1	0.88	-167.44	0.98	229.39	62.47	93	178	232	254	379
	Instar 2	0.89	-179.23	1.02	280.78	85.06	92	207	278	310	455
	Instar 3	0.89	-173.57	1.05	315.87	83.13	129	240	309	341	467
	Instar 4	0.89	-182.26	1.01	349.63	68.01	200	291	349	374	494
	Instar 5	0.93	-206.76	0.98	423.54	50.25	314	382	425	443	543
	Stem boring stage*	0.94	-236.43	1.01	260.81	82.28	80	191	260	329	438
	Crown feeding stage	0.91	-206.21	1.02	356.66	78.02	184	288	354	418	514
	Adult	0.71	-151.79	0.97	812.83	140.03	510	700	821	871	1170
Second generatio	All larvae	0.75	-166.48	1.00	956.88	152.95	620	826	955	1012	1286
	Instar 1	0.58	-105.47	0.93	894.89	154.27	568	780	917	968	1401
	Instar 2	0.79	-107.87	0.95	919.88	102.50	700	840	930	965	1207
	Instar 3	0.91	-103.13	1.03	1051.99	118.26	789	947	1046	1091	1283
	Instar 4	0.66	-89.04	0.89	1014.93	119.16	768	933	1043	1081	NA
	Instar5	0.94	-121.24	0.97	1129.17	110.11	891	1041	1136	1175	1413
	Adult	0.72	-78.49	1.00	1432.44	119.95	1169	1330	1432	1476	1693

* Stem boring stage consists of instar 1 and 2. ** Crown feeding stage consists of instar 3, 4, and 5.

Fig. 1. Nonlinear model of cumulative emergence of *L. maculicollis* overwintered adults as a function of accumulated GDD_0 (solid line) with 95% confidence band (dashed lines). Symbols are observed data at each site in each year.

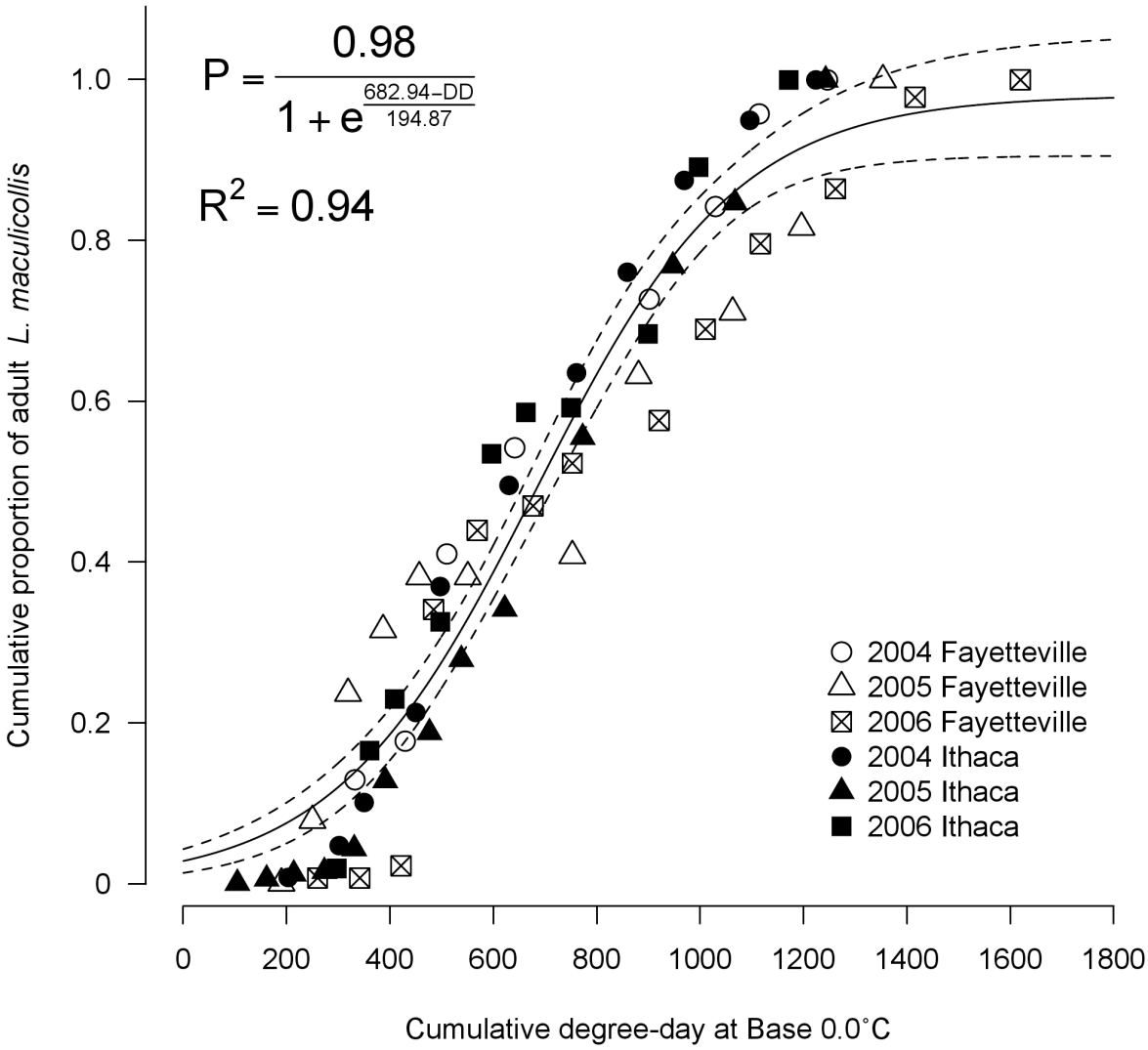

$P = \frac{0.98}{1 + e^{\frac{682.94 - DD}{194.87}}}$
$R^2 = 0.94$
Cumulative proportion of adult *L. maculicollis*
0
0.2
0.4
0.6
0.8
1.0
0
200
400
600
800
1000
1200
1400
1600
1800
Cumulative degree-day at Base 0.0˚C
2004 Fayetteville
2005 Fayetteville
2006 Fayetteville
2004 Ithaca
2005 Ithaca
2006 Ithaca

Fig. 2. Nonlinear model of cumulative occurrence of *L. maculicollis* larvae (all instars combined) from the first generation as a function of accumulated $GDD_{7.2}$ (solid line) with 95% confidence band (dashed lines). Symbols are observed data at each site in each year.

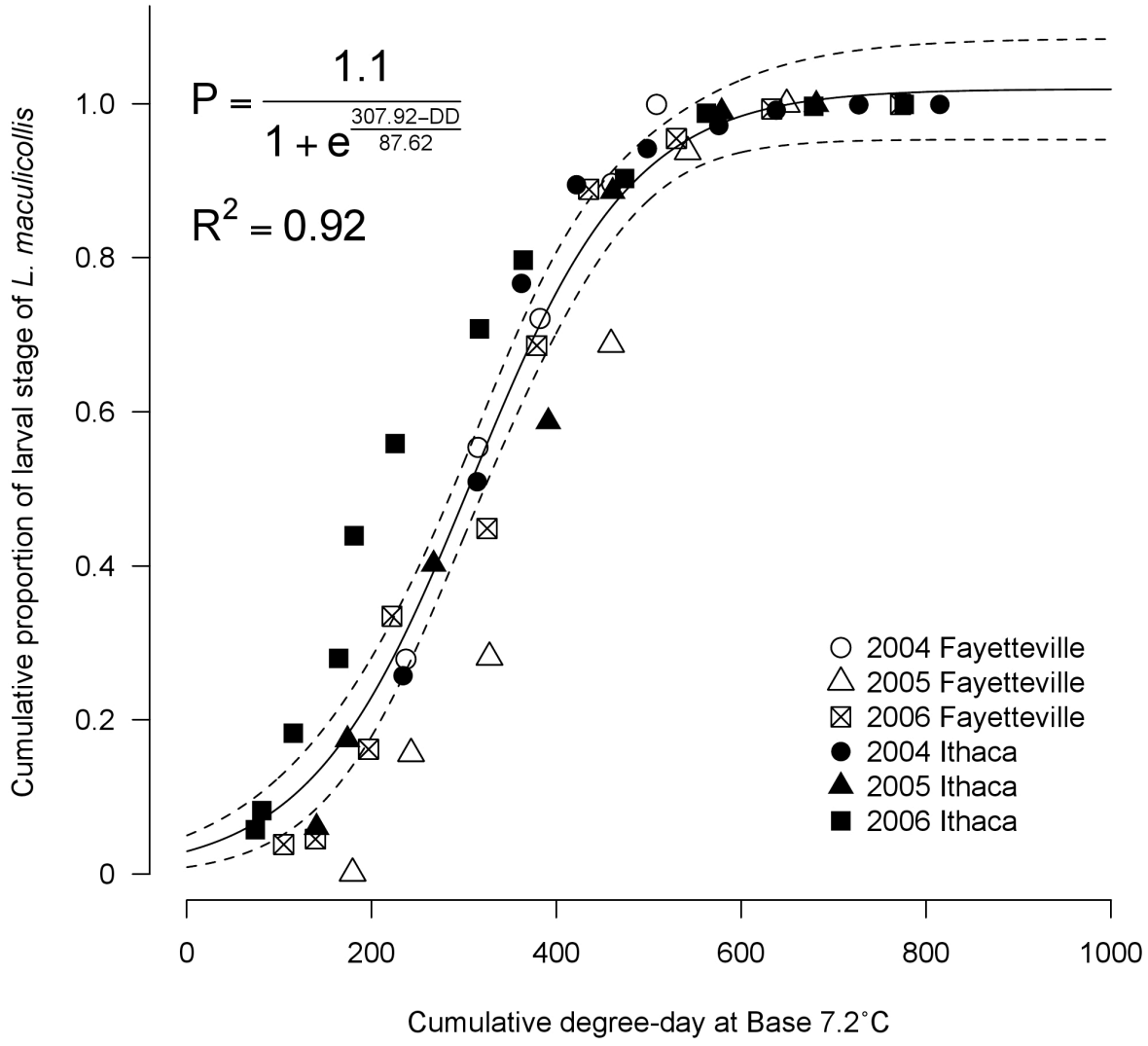

$P = \frac{1.1}{1 + e^{\frac{307.92 - DD}{87.62}}}$
$R^2 = 0.92$
Cumulative proportion of larval stage of *L. maculicollis*
0
0.2
0.4
0.6
0.8
1.0
0
200
400
600
800
1000
Cumulative degree-day at Base 7.2˚C
2004 Fayetteville
2005 Fayetteville
2006 Fayetteville
2004 Ithaca
2005 Ithaca
2006 Ithaca

Fig. 3. Nonlinear model of cumulative occurrence of stem boring stage of *L. maculicollis* larvae from the first generation as a function of accumulated $GDD_{7.2}$ (solid line) with 95% confidence band (dashed lines). Symbols are observed data at each site in each year.

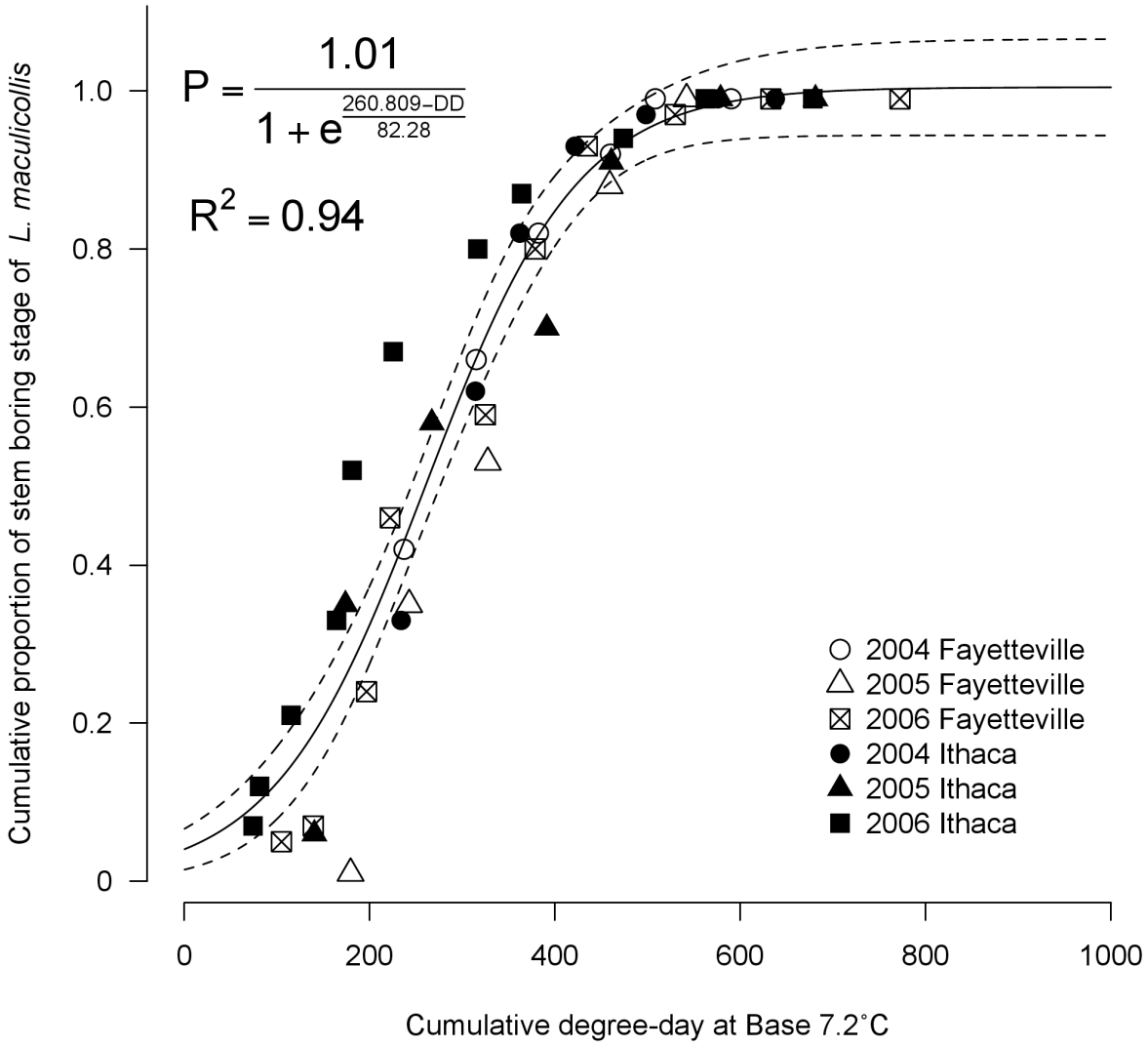

P = 1.01 / (1 + e^((260.809−DD)/82.28))
R² = 0.94
Cumulative proportion of stem boring stage of L. maculicollis
Cumulative degree-day at Base 7.2˚C
0
0.2
0.4
0.6
0.8
1.0
0
200
400
600
800
1000
2004 Fayetteville
2005 Fayetteville
2006 Fayetteville
2004 Ithaca
2005 Ithaca
2006 Ithaca

Fig. 4. Nonlinear model of cumulative occurrence of crown feeding stage of *L. maculicollis* larvae from the first generation as a function of accumulated $GDD_{7.2}$ (solid line) with 95% confidence band (dashed lines). Symbols are observed data at each site in each year.

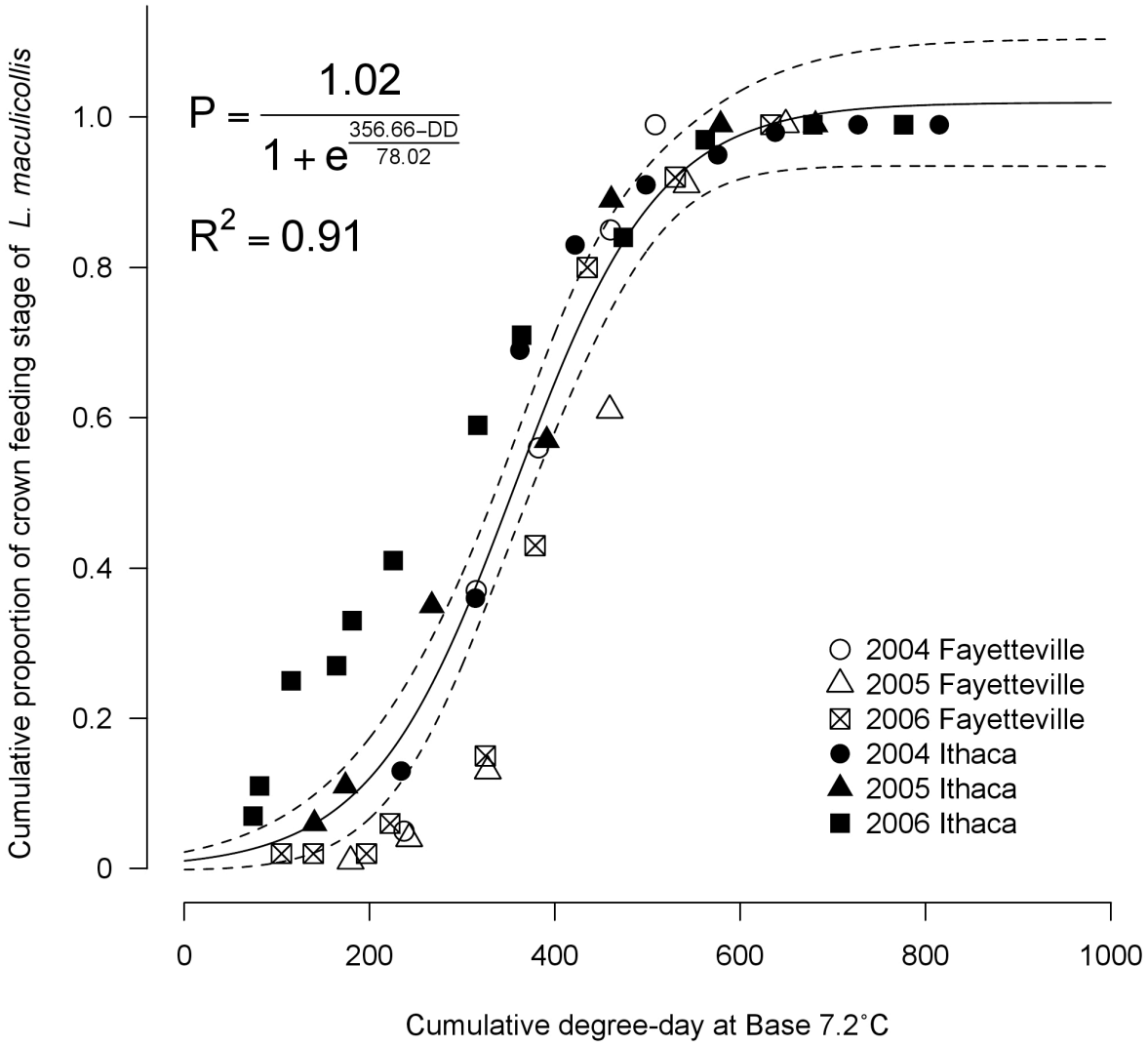
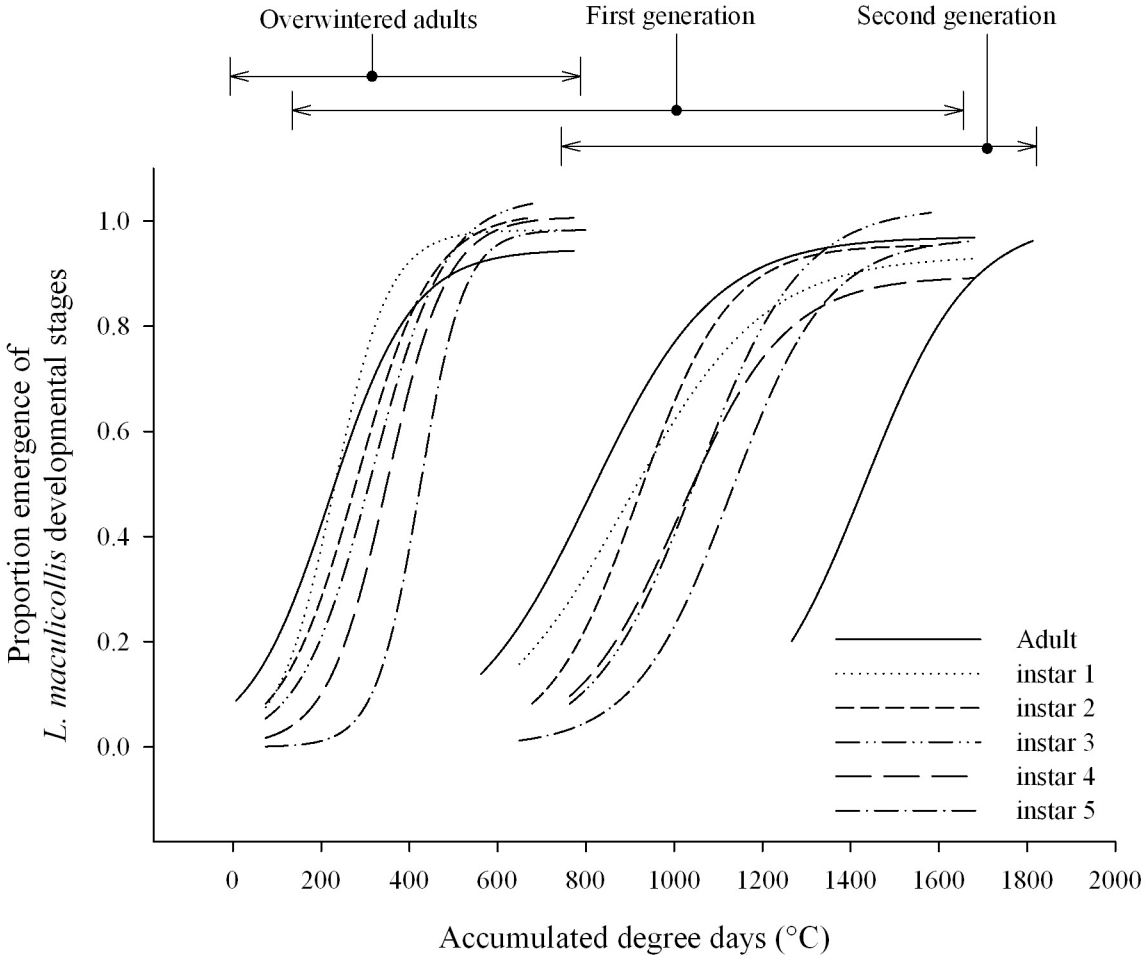

P = 1.02 / (1 + e^((356.66−DD)/78.02))
R² = 0.91
Cumulative proportion of crown feeding stage of L. maculicollis
Cumulative degree-day at Base 7.2˚C
0
0.2
0.4
0.6
0.8
1.0
0
200
400
600
800
1000
2004 Fayetteville
2005 Fayetteville
2006 Fayetteville
2004 Ithaca
2005 Ithaca
2006 Ithaca

Fig. 5. Nonlinear models with base temperature 7.2˚C of cumulative emergence of *L. maculicollis* developmental stages through season. Arrow lines indicate the start and end of each generation.


Overwintered adults
First generation
Second generation
Proportion emergence of
L. maculicollis developmental stages
1.0
0.8
0.6
0.4
0.2
0.0
Adult
instar 1
instar 2
instar 3
instar 4
instar 5
0
200
400
600
800
1000
1200
1400
1600
1800
2000
Accumulated degree days (°C)

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CHAPTER TWO

VALIDATION OF A DEGREE-DAY MODEL FOR *LISTRONOTUS MACULICOLLIS* KIRBY (COLEOPTERA: CURCULIONIDAE) DEVELOPMENT IN NEW YORK GOLF COURSES

ABSTRACT

Field studies were conducted over two seasons to validate a nonlinear degree-day model for cohort development of *Listronotus maculicollis* Kirby (Coleoptera: Curculionidae) whose larvae cause severe damage to golf course turf in the Northeast U.S. First and second instars reside and feed inside the host plant, and third, fourth and fifth instars reside in the topsoil and feed on crowns. These five larval stages were divided into two groups, stem boring (first, and second instars) and crown feeding (third, fourth and fifth instars), and field-collected abundance data for both groups was compared to predictions based on a degree-day model generated from population data collected in central NY for three years prior to this validation study. Larval population data for validation were collected over two years at seven golf courses across NY, which belong to USDA hardiness zones 5a to 7a. In both 2008 and 2009, the observed cumulative proportional emergence of the stem boring stage consistently preceded the predicted values and the majority of the data points fell outside of 95% confidence intervals. For the crown feeding stage, the data points similarly preceded the predicted values except for those collected from one site, which showed the development as predicted. The population accumulation rates of each stage were much faster than those of previously collected population data and the model prediction was off by a maximum of twenty-two days. However, the three-parameter logistic equation fitted to each data set of the current study describes the pattern or shape of the population accumulation curve for each site rather well ($R^2 > 85$). Further investigations into

factors causing differences in population developmental rates of *L. maculicollis* in different geographical areas are required to improve the model.

INTRODUCTION

Listronotus maculicollis Kirby (Coleoptera: Curculionidae) whose larvae cause severe damage to golf course turf, is a native weevil pest of highly maintained cool season turfgrasses in the Northeastern United States and Southeastern Canada (Vittum et al. 1999). The damage is most apparent in low-mown areas of golf courses (e.g. fairways, greens, tees) with high percentages of *Poa annua* L. The geographical distribution of damage from *L. maculicollis* populations has recently expanded from its recorded epicenter around the New York City metropolitan area to the entire New England region, south to Virginia, west to Ohio, and north to Quebec (Simard et al. 2007).

Major efforts to prevent *L. maculicollis* damage are based on applying broad-spectrum insecticides to control adults moving from overwintering sites in spring to low-mown developmental sites prior to oviposition, and/or the larvae before the appearance of visual damage. Nevertheless, all the efforts tend to be in vain due to the difficulty in timing insecticide applications to target the insect between the extended emergence periods of overwintering adults and the extended developmental periods of larvae (Diaz et al. 2007; Diaz et al. 2008; McGraw and Koppenhöfer 2009). Without effective monitoring methods, multiple insecticide applications are commonly made against a single generation and the repeated use of synthetic insecticides, especially pyrethroids, has led to the development of insecticide resistance in some populations (Ramoutar et al. 2009).

To time insecticide applications against adults, golf course superintendents commonly rely on plant phenological cues that suggest an insecticide application sometime between full

bloom of *Forsythia* spp. and full bract of *Cornus florida* L. (Tashiro et al. 1978). This strategy, which is based on anecdotal observations, does not consistently provide reliable timing. Moreover, there is no timing strategy available to target larvae in the field. Early-stage larvae of *L. maculicollis* feed within the host plant (stem boring stage), and hence, are protected from most chemical insecticides. Late-stage larvae feed externally on the crown, the active growth point of the plant, and cause the most severe damage to the turf (crown feeding stage) (Vittum et al. 1999; Vittum 2005). For effective control of larval stages, it is critical to understand the difference in developmental patterns of larvae in the plants and in the surface soil. Thus, to accurately predict phenology and timing of management actions, a monitoring tool for *L. maculicollis* that is based on a common developmental and/or behavioral factor is needed.

Since development of ectothermic animals such as insects depends on ambient temperature and time (Davidson 1944; Allen 1976; Bonhomme 2000), degree-day (DD) models are useful tools in ecology and pest management to monitor the development and phenology of insects in the field. Seto and Peck (submitted) developed DD models for *L. maculicollis* developmental stages and the cumulative emergence/development of overwintered adults, larvae of the stem boring stage, and larvae of the crown feeding stage (R^2 = 0.91-0.94). The models were constructed from population data collected at two golf courses in New York. It is unknown whether the models are applicable to other golf courses across NY, where the climate patterns vary. Thus, in this study, we evaluated the validity of the models across NY based on the assumption that the timing of adult movement in the spring and peak occurrence of the different developmental stages of *L. maculicollis* populations in different geographical areas are linked by a common DD model. Due to the limits of sampling labor, the emphasis was put on larval stages.

MATERIALS AND METHODS

Sites

Surveys were conducted over two years on a total of seven golf courses in NY with histories of *L. maculicollis* infestations (Table 1). In 2008, larval populations of *L. maculicollis* were collected from five golf courses: Lake Shore Yacht and Country Club, Cicero (43.196231, -76.050191), Otterkill Golf and Country Club, Campbell Hall (41.463095, -74.226769), Onondaga Golf and Country Club, Fayetteville (43.028254, -76.040905), Robert Trent Jones Golf Course, Ithaca (42.457723, -76.464262), and Dunwoodie Golf Course, Yonkers (40.933216, -73.87624). In 2009, larval populations were collected from seven sites: Lake Shore Yacht and Country Club, Otterkill Golf and Country Club, Onondaga Golf and Country Club, Robert Trent Jones Golf Course, Maple Moor Golf Course, White Plains (41.001939, -73.727324), and Pine Hollow Country Club, East Norwich (40.851379, -73.522465). The golf courses are in USDA hardiness zones 5a to 7a (Table 1). At each golf course, we marked four 2 m by 20 m strips on the periphery of fairways. The locations of the strips were selected based on the history of previous infestations and the similar level of sun exposure. Strips on the same golf course were separated by a minimum of 50 m. The grasses on the fairways were mowed at approximately 1.25 cm. Each strip was 10 to 20 m from a tree line where previous research indicated a primary area of adult overwintering (Diaz and Peck 2007). No pesticides were sprayed over the strips during the experiment.

Larval sampling and extraction

The larval sampling commenced before the emergence of larval stages was observed. The sampling period started between 24 and 30 April in 2008 and between 28 March and 14 April in 2009, with variation due to the difference in the rate of DD accumulations at each site. Weekly, within each strip, twenty 6-cm diameter by 6.4-cm depth soil cores were removed with a turf-corer (TTP1-M Turf-Tec Tubular Turf Plugger, Turf-Tec International, Tallahassee, FL) every 1

m along the length of the sampling strip. The cores were gathered in a plastic bag, sealed and immediately transported to the maintenance shop at each golf course. In the maintenance shop, a portable heat extraction system, which was modified from the heat extraction unit developed by Diaz et al. (2008), was set up prior to the sampling. The portable system consisted of four plastic containers, a four-tiered metal rack, a 30-pint capacity commercial dehumidifier and a plastic sheet large enough to cover the entire unit. A 23 cm by 28 cm yellow sticky sheet (AR914 Yellow Sticky Trap, ISCA TECHNOLOGIES, Inc., Riverside, CA) was placed at the bottom of the plastic container (L 30 cm x W 25 cm x H 10 cm) and1-cm welded stainless mesh was fixed at the height of 5 cm from the bottom. Each set of twenty soil cores was placed with grass-side facing down on the mesh and the prepared plastic containers were placed in the metal rack. With the dehumidifier running, the rack was wrapped with a plastic sheet. Temperature inside of the unit stayed between 30-35 ˚C and larvae were extracted from soil cores and captured onto the yellow sticky sheet. The extraction was continued for 2-3 days until the soil cores thoroughly dried. The collaborators at each location immediately recovered the sticky sheets, wrapped each with a cling wrap and mailed them to the Geneva, NY laboratory. All larvae captured on the sticky sheets were identified to larval instar by measuring the head-capsule width (Diaz et al., 2008) through microscopic examinations. The count data of first and second instars were grouped as stem boring larvae, and those of the third, fourth and fifth instars were grouped as crown feeding larvae. Weekly sampling ended when the fifth instars were no longer observed in the population, indicating the end of the larval stage of the first generation (8 July – 4 August in both years).

Model validation

In the previous study (Seto and Peck, submitted), the authors developed a three-parameter nonlinear DD model for *L. maculicollis* population development of different life-cycle stages

collected at two golf courses in NY. The nonlinear logistic function was as follows:

$$P = \frac{\alpha}{1 + e^{(\frac{\beta - DD}{\gamma})}}$$

where P is the cumulative fraction of *L. maculicollis* developmental stage, α is the horizontal asymptote (expected to be approximately 1), β is the value of DDs at the inflection point, and γ determines the slope at inflection (the lower γ gives the steeper slope). We explored the validity of this model for different sites in NY over two field seasons based on the following procedures: (1) Overlaying the model predictions (with confidence intervals) with observed numbers of stem boring and crown feeding stages obtained in the current study); (2) The three-parameter logistic curves were fitted to each set of population data from the current study and the coefficients of the models were compared with those of the original model; (3) We determined that the model was acceptable as a prediction tool when the prediction was in the range of ± 7 days of observed data. This acceptable range was determined based on the model construction process, in which 14 days was the maximum separation between one observation and another. A nonparametric method was used to statistically validate the model; the model's mean square prediction error (MSPE) was compared to mean square error for the model (MSE_{model}) (Kutner et al. 2004). The effect of the number of days diverging from the model was compared to MSPE by adding or subtracting 10.56 DD to the model predictions for stem boring larvae and 10.64 DD for crown feeding larvae (these values were the average DD accumulation per day in the data set used to generate the original models). By using this value, calibrated $MSPE/MSE_{model}$ ratios that would arise from data within the acceptable range (± 7 days) were calculated. The model was determined valid if the ratio of calculated $MSPE/MSE_{model}$ was smaller than the calibrated ratio. If the ratio was higher than the calibrated ratio (Table 3), we determined the model was significantly biased. (4) Observed cumulative DDs and Julian date for 50% population

accumulation of *L. maculicollis* larvae were also compared to the predicted values calculated from the previously developed model.

Temperature data (daily maximum and minimum) were obtained from on-site weather stations at each golf course. On the occasion of encountering missing temperature data, the gaps were filled with data from the nearest National Weather Service Station. DDs were estimated as follows (Arnold, 1960):

$$\text{Cumulative DDs} = \sum \left[\frac{(T_{max} + T_{min})}{2} - (T_{base}) \right]_n$$

where T_{max} is the daily maximum temperature, T_{min} is the daily minimum temperature, T_{base} is the lower developmental threshold temperature below which growth processes cease, and n is the day number. T_{base} was set at 7.2˚C in this study. Negative DDs were assigned values of zero.

Regression analysis and graphing were conducted using R, version 2.14.0 (R Development Core Team 2011).

RESULTS

Larval *L. maculicollis* population data were successfully obtained from five of seven sites (Table 2). The data from Fayetteville and Yonkers were too low and fragmented to describe the development of larval populations. The data for the crown feeding stage in East Norwich were incomplete due to an accidental application of insecticide over the sampling strips and, thus, excluded from the analysis.

The observed data for stem boring larvae, for the most part, did not fall within the confidence intervals of the original model (Fig. 1). Stem boring larvae from all the sites generally showed faster accumulation than the model prediction. The value of DD at the inflection point (*β,)*, from the model fitted to stem boring larvae collected at each site was

consistently lower than that of the model (Table 2). The data from Campbell Hall in 2008 had a calculated β value that was only half of the model's predicted β and showed the steepest slope (γ = 15.62), which indicated the stem boring larvae in Campbell Hall in 2008 reached the 50 % accumulation almost 14 days earlier than model prediction if the average daily DD accumulation was assumed as 10.56 DD. Using ± 7 days for an acceptable prediction range (Table 3), $MSPE/MSE_{model}$ ratios for each site per year indicated the model gave reasonable predictions only for the stem boring larvae from Cicero in 2008 and from East Norwich in 2009 (Table 2).

The observed data for crown feeding larvae at Cicero in 2008 and 2009 fell between the confidence intervals of the model (Fig. 2). Crown feeding larvae from the other sites showed faster accumulation than the model prediction, as was found with the stem boring larvae. Similar to the stem boring larvae, the sample from Campbell Hall in 2009 had a β value that was a half of the model's and a γ value that was the smallest among the samples, which indicated the crown feeding larvae in Campbell Hall in 2008 reached the 50 % accumulation almost 17 days earlier than model prediction if the average daily DD accumulation was assumed as 10.60 DD. With ± 7 days of acceptable prediction range (Table 3), $MSPE/MSE_{model}$ for each site per year indicated the model for crown feeding larvae provided a reasonable prediction for the data from Cicero and Ithaca in both years (Table 2).

The differences between observed and predicted DDs at 50% population accumulation of stem boring larvae (Observed - Predicted) were all negative, which indicated the predicted DD was later than the observed in all sites and years, and ranged from -36.0 to -123.6 DD (Table 4). Differences as measured by Julian Date (JD) ranged from -4 to -22, which indicated the predicted JD for 50% population accumulation of stem boring larvae was 4 to 22 days later than the observed JD. At two sites where the model predictions were within the ± 7 days of acceptable prediction range, the difference resulted in -12 JD (Cicero 2008) and -4 JD (East Norwich 2009).

For 50% population accumulation of crown feeding larvae, the difference between observed and predicted DDs ranged from +13.7 to -176.3 (Table 5). In terms of JD, the model gave perfect predictions for the data from Cicero in both years. At the two sites where the model predictions were in the acceptable prediction range, the difference resulted in -8 JD (Ithaca 2008) and -10 JD (Ithaca 2009).

DISCUSSION

The main goal of this study was to evaluate the validity of a DD model predicting the timing of peak occurrence of the different developmental stages of *L. maculicollis* populations in different geographical areas across NY that belonged to different USDA hardiness zone from 5a to 7a. Previous population studies, as well as the current study, showed significant variation in the timing of peak occurrence of *L. maculicollis* developmental stages (Vittum and Tashiro 1987; Simard 2007; Diaz et al 2008), as great as two weeks even in the same year within geographically adjacent (~ 70 km) sites. To potentially explain this variation based on temperature differences, we developed new DD models to standardize the variation in population accumulation over time to temperature measures. We expected that the original model would predict the population accumulation patterns of the beetle despite the different climate ranges from which the tested population data were collected. However, our results showed instead that the observed population accumulations at each accumulated degree-days during the experiment preceded the predicted time, for both stages at almost all sites. The variation chiefly arose from the unexpectedly fast population accumulation rate that exceeded the model prediction at the all sites (Table 2). It is interesting to note that the population accumulation data collected in Ithaca in the present study, one of the sites used to construct the original model, was not very well described by the model (Figs. 1 and 2).

Host plant availability is a major factor for *L. maculicollis* development. Rothwell (2003) reported that larvae feeding on *P. annua* obtained greater weight compared to feeding on other grass species such as creeping bentgrass, *Agrostis stolonifera*, Host plant availability, thus, could be a major factor for beetle development. *P. annua* densities, however, were more than 85% of grasses in all the sites in the current study and it was unlikely to affect the population accumulation rate to such a degree as to invalidate the model predictions. The host plant has two major biotypes that are commonly found on golf courses in the Northeast: annual biotypes, *P. annua annua*, and perennial biotypes, *P. annua reptans* (Turgeon 2008). There are also intermediate biotypes that are somewhere between P. *annua* and P. *reptans*. Our preliminary study on field-collected *L. maculicollis* from Ithaca, its growth on different biotypes of *P. annua* indicated different developmental rates under the same laboratory conditions (data not shown). Hence, in future studies the influence of *P. annua* biotype on growth response of *L. maculicollis* should be taken in account. In addition to host plant biotypes, insect biotypes might be another factor causing the observed variation (Via, 1990; Delatte et al., 2009). Currently little information exists on genetic variation in *L. maculicollis* in terms of development or other ecologically relevant variables.

Phenotypic plasticity, an environmentally induced phenotypic change, has been considered as a major strategy that maximizes or retains fitness in an organism inhabiting variable environments (Via and Lande 1985; Schlichting and Pigliucci 1998; Pigliucci 2005). Rather than the norm, extremes of environmental variables are considered to exert selective pressures on ectotherms and certain life-history traits can exhibit profound variations as a consequence (Stevens 2004; Kingsolver and Huey 2008). We know little about the effect of extreme temperatures on development of the beetle. Moreover, there is no information to determine what temperature becomes extreme for the beetle. These aspects should also be

explored in the future.

Climatic regions (USDA hardiness zone, Table 1) might have considerable effects on the development of *L. maculicollis*. Obrycki and Tauber (1982) showed that four out of five species of coccinellid beetles collected in the same climatic region (Ithaca, NY) shared a similar lower temperature developmental threshold, but the number of degree-days to complete development differed from species to species. In different climatic regions, a given species (aphidophagous coccinellid, chrysopid, hermerobiid, or syrphid) showed an ability to adjust the lower developmental threshold to adapt to local habitat conditions, resulting in considerable variation observed in the lower developmental threshold between different populations of the same species (Honěk and Kocourek 1988). If this applies to *L. maculicollis*, we need to determine the lower developmental thresholds for different populations in order to define the areas that are the same. This would allow construction of a site-specific population predictive model. However, the sensitivity of the model to the lower developmental thresholds was low based on the range of temperatures tested for the lower developmental threshold (0, 4.4, 7.2, 10.0, 13.3 ˚C) in the model construction (Seto and Peck submitted). The different thresholds caused little variation for the values of Akaike's Information Criteria, which was used for the model selection.

Different climatic regions are also related to other abiotic/biotic factors, especially photoperiod, and it is unknown how *L. maculicollis* development is affected by this important cue. Goldson (1979a,b; 1981) determined in a laboratory study that photoperiod is the most important factor controlling the reproductive dormancy of *L. bonariensis*, a species closely related to *L. maculicollis*. Lopatina et al (2011) showed that an insect's ability to adjust the lower developmental threshold to adapt to the local environmental conditions occurs in the course of the season and is driven by the changes in photoperiod. The major local environmental conditions *L. maculicollis* typically experience in spring is a decline of the food quality. *P. annua*

and other cool-season turfgrasses typically show a bimodal growth pattern, in which, after the vigorous spring flush, the shoot growth gradually slows toward summer and they may even stop growing all together during summer (Turgeon 2008). First generation adults show up around the time of year shoot growth is at its lowest., The beetle might reduce its lower developmental threshold in response to the declining quality of food and longer photoperiod in order to complete development around the same time regardless of when they hatched.

In conclusion, the previously proposed three-parameter logistic model did not consistently provide practical predictions of *L. maculicollis* population accumulation across NY. The model did, however, describe the pattern or shape of the population accumulation curve for each site well ($R^2 > 85$, Table 2). With the three parameters, the site-specific models could be used for predicting development, with the assumption that the population accumulation patterns do not fluctuate drastically in the corresponding area. To improve the model, we need to understand those factors causing the comparable differences in population accumulation rates of *L. maculicollis* in different geographical areas. Studies on the relationship between beetle development and abiotic/biotic factors in both microhabitat and macro scale might contribute to this understanding and then allow for selection of more accurate model parameters.

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Table 1. Decimal degrees of study sites and the USDA hardiness zone to which the sites belong.

Site	Latitude degree (°)	Longitude degree (°)	U SDA hardiness zone	Survey year
Campbell Hall	41.4631	-74.2268	5b	2008, 2009
Cicero	43.1962	-76.0502	5a	2008, 2009
East Norwich	40.8514	-73.5225	7a	2009
Fayetteville	43.0283	-76.0409	5a	2008, 2009
Ithaca	42.4577	-76.4643	5a	2008, 2009
White Plains	41.0019	-73.7273	6b	2009
Yonkers	40.9332	-73.8762	6b	2008

Table 2. The coefficients of nonlinear logistic models with base temperature 7.2 for *L. maculicollis* larval stages. Nonlinear model $P=\alpha/(1+\exp((\beta-DD)/\lambda))$ where P is cumulative proportion of *L. maculicollis* captured, α,β, and γ are model coefficients, and DD is the independent variable (DD).

		Stem boring stage				Crown feeding stage			
Site	Year	α	β	γ	MSPE/MSE_{model}*	α	β	γ	MSPE/MSE_{model}*
Model	-	1.01	260.89	82.28	-	1.02	356.66	78.02	-
Campbell Hall	2008	0.98	135.90	15.62	12.17	0.96	175.13	25.49	3.68
	2009	1.01	140.29	26.98	11.09	0.97	207.93	47.13	2.83
Cicero	2008	0.94	209.31	30.84	**1.37**	1.00	367.30	62.39	**0.61**
	2009	0.97	181.51	19.92	2.73	0.98	351.39	76.07	**0.76**
East Norwich	2009	0.96	222.23	23.78	**1.57**	-	-	-	-
Fayetteville	2008	-	-	-	-	-	-	-	-
	2009	-	-	-	-	-	-	-	-
Ithaca	2008	0.98	191.57	16.95	3.07	0.94	239.84	28.14	**1.13**
	2009	0.97	177.79	54.02	1.66	0.99	276.44	34.30	**1.40**
White Plains	2009	0.86	206.22	16.51	1.91	0.91	238.32	26.33	1.67
Yonkers	2008	-	-	-		-	-	-	-

* MSPE: Mean square prediction error, MSE_{model}: Mean square error for the model. The MSPE/MSE_{model} ratios within ±7 days acceptable ranges were shown in bold.

Table 3. $MSPE/MSE_{model}$ ratios for early/late calibration for *L. maculicollis* larval stages.

	MSPE/MSE	
calibration	Stem boring	Crown feeding
-10	2.54	2.32
-9	2.18	2.03
-8	1.87	1.79
-7	**1.60**	**1.57**
-6	**1.38**	**1.39**
-5	**1.17**	**1.24**
-4	**1.08**	**1.12**
-3	**0.99**	**1.04**
-2	**0.95**	**1.00**
-1	**0.95**	**0.98**
1	**1.09**	**1.05**
2	**1.23**	**1.14**
3	**1.41**	**1.26**
4	**1.64**	**1.41**
5	**1.92**	**1.60**
6	**2.23**	**1.82**
7	**2.60**	**2.07**
8	3.00	2.36
9	3.46	2.68
10	3.95	3.04

*±7 days acceptable ranges were shown in bold.

Table 4. Validation of the DD model predicting 50% accumulation of the stem boring stage of *L. maculicollis* at golf courses in NY

		DD at 50% population accumulation			JD at 50% population accumulation		
Site	Year	Observed	Predicted	Difference*	Observed	Predicted	Difference*
Campbell Hall	2008	136.5	260.1	-123.6	127	149	-22
	2009	140.0	260.1	-120.0	126	144	-18
Cicero	2008	213.4	260.1	-46.7	134	146	-12
	2009	182.9	260.1	-77.2	135	144	-9
East Norwich	2009	224.1	260.1	-36.0	128	132	-4
Ithaca	2008	192.1	260.1	-67.9	139	154	-15
	2009	181.1	260.1	-78.9	140	148	-8
White Plains	2009	211.6	260.1	-48.4	125	130	-5

* Difference was obtained by subtracting predicted values from observed values.

Table 5. Validation of the DD model predicting 50% accumulation of the crown feeding stage of *L. maculicollis* at golf courses in NY

		DD at 50% population accumulation			JD at 50% population accumulation		
Site	Year	Observed	Predicted	Difference*	Observed	Predicted	Difference*
Campbell Hall	2008	177.3	353.6	-176.3	136	157	-21
	2009	210.8	353.6	-142.8	137	155	-18
Cicero	2008	367.3	353.6	13.7	157	157	0
	2009	354.2	353.6	0.6	157	157	0
Ithaca	2008	243.7	353.6	-109.9	152	160	-8
	2009	277.4	353.6	-76.2	151	161	-10
White Plains	2009	243.7	353.6	-109.9	128	141	-13

* Difference was obtained by subtracting predicted values from observed values.

Fig. 1. Prediction line (solid line) and 95% confidence band (dashed lines) of nonlinear model of the cumulative proportion of stem boring stage of *L. maculicollis* larvae from the first generation versus cumulative degree-day at base 7.2˚C plotted with the observed data from validation study sites.

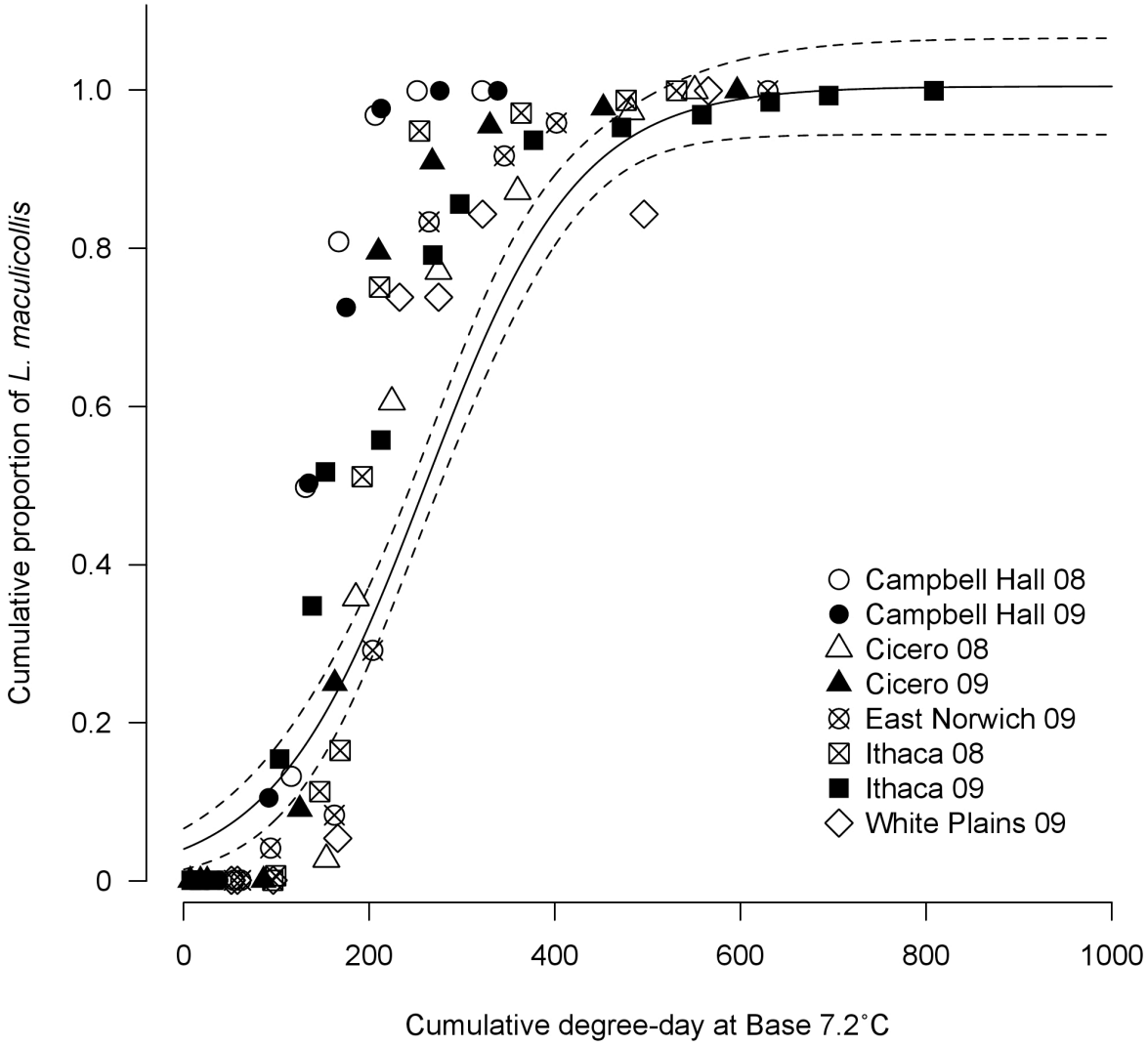
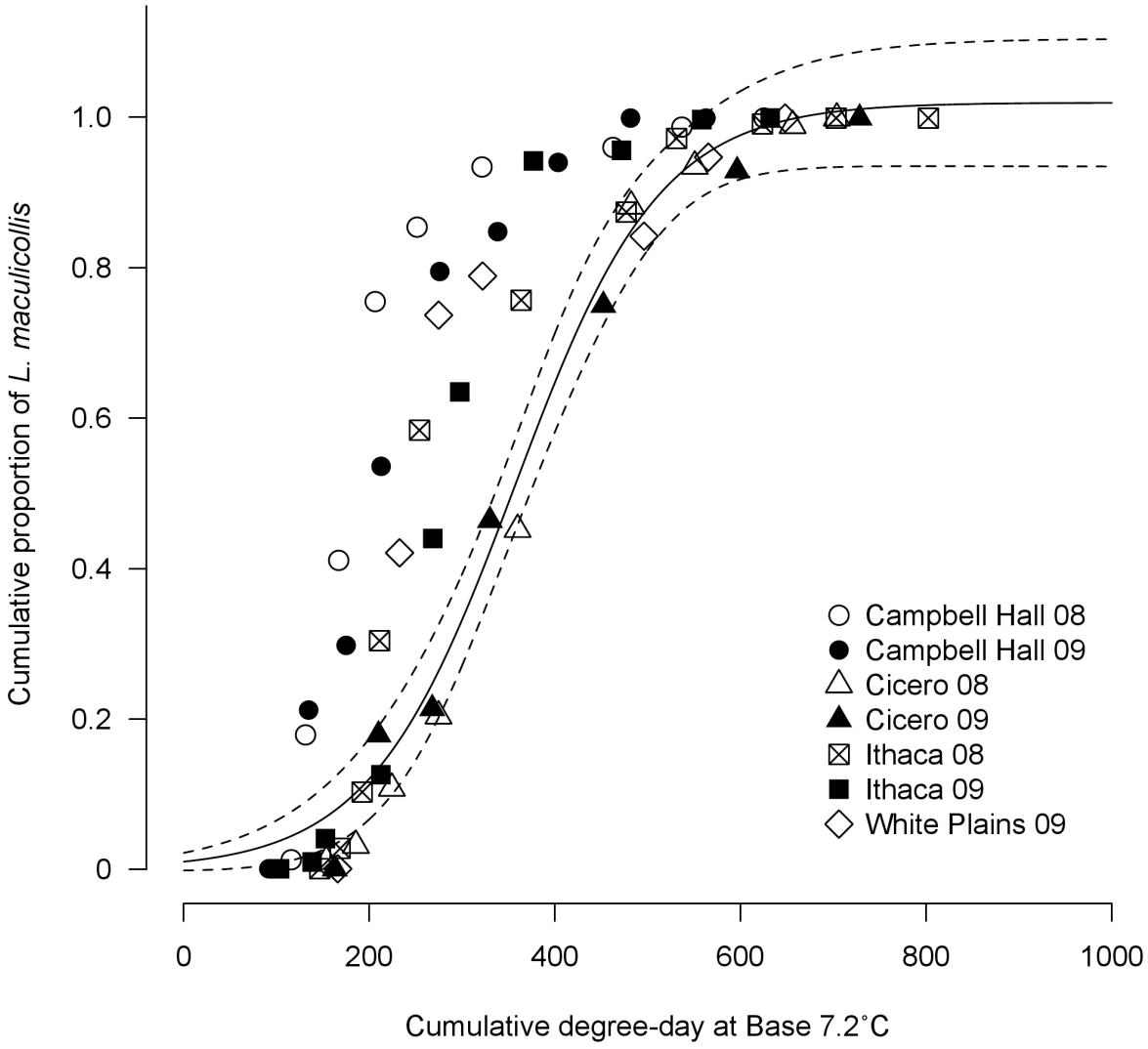

Cumulative proportion of L. maculicollis
1.0
0.8
0.6
0.4
0.2
0
0
200
400
600
800
1000
Cumulative degree-day at Base 7.2˚C
Campbell Hall 08
Campbell Hall 09
Cicero 08
Cicero 09
East Norwich 09
Ithaca 08
Ithaca 09
White Plains 09

Fig. 2. Prediction line (solid line) and 95% confidence band (dashed lines) of nonlinear model of cumulative proportion of crown feeding stage of *L. maculicollis* larvae from the first generation versus the cumulative degree-day at base 7.2˚C plotted with the observed data from validation study sites.


Cumulative proportion of L. maculicollis
1.0
0.8
0.6
0.4
0.2
0
0
200
400
600
800
1000
Cumulative degree-day at Base 7.2˚C
Campbell Hall 08
Campbell Hall 09
Cicero 08
Cicero 09
Ithaca 08
Ithaca 09
White Plains 09

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CHAPTER THREE

DYNAMICS OF SPATIOTEMPORAL DISTRIBUTIONS OF *LISTRONOTUS MACULICOLLIS* KIRBY (COLEOPTERA: CURCULIONIDAE) IN A NEW YORK GOLF COURSE

ABSTRACT

The spatial and temporal dynamics of *Listronotus maculicollis* Kirby (Coleoptera: Curculionidae) populations, a destructive pest of close-cut turf in golf courses of the Northeast, were examined. Larval stages that feed on stems and crowns of the host plant have been determined to be the primary cause of damage; however, previous studies have not supported a consistent association between larval density and damage severity. In order to better correlate damage to larval presence, we investigated the spatial associations among insect density (adult and larva), host plant density and habitat variables (soil moisture, soil nutrients, thatch depth, etc.). Spatially-referenced data were sampled weekly on a golf course fairway in NY over a 2yr period. Spatio-temporal dynamics of the life stages and spatial associations between variables were analyzed by SADIE (Spatial Analysis by Distance IndicEs). Damage expression was significantly associated with larval density, thatch depth, and sand, while it was not significantly associated with host plant density. The results show that percent of damage due to larval feeding becomes more severe under conditions of deep thatch and high soil nitrate concentration. While no cultural control tactics are currently used to suppress *L. maculicollis* damage, we propose that agronomic practices affecting thatch accumulation and nutrient levels should be explored for ABW management on golf course turf.

INTRODUCTION

Spatial variation of insect pests is a fundamental component of population dynamics. Measuring and understanding this variation provides opportunities to exercise spatially variable pest management. Site-specific pest management practices may not only reduce the use of insecticides but also slow the rate of resistance development by providing temporal refuges (Weisz et al. 1996; Midgarden et al. 1997). To achieve efficient and effective pest management by targeting pests accurately in space, it is essential to describe the spatiotemporal distribution and elucidate the underlying mechanisms of the target pest.

Listronotus maculicollis Kirby (Coleoptera: Curculionidae) is the most damaging insect pest of *Poa annua* L., a common grass species managed as turf on golf courses throughout the Northeastern United States (U.S.) (Vittum et al. 1999). The weevil normally has two generations a year in the Northeast; the first generation is mainly associated with damage (McGraw and Koppenhofer, 2007). The beetles overwinter as adults in the leaf litter and tall grasses that are within the range of tens to hundreds of meters away from the short-mown turf, such as fairways, tees and greens, where they will reproduce and develop during the season (Vittum, 1980; Diaz and Peck, 2007). The immigration of the beetle into the short-mown turf areas was evidenced by observing walking adults in the early spring (Diaz and Peck, 2007). Although adults presumably feed on grass and reproduce upon encountering short-mown turf, there is little to no information about the spatial patterns of the colonization on golf course breeding sites by *L. maculicollis* overwintered adults. We also do not have information about the spatial dynamics of the establishment of the larval stages following the adults.

The lack of information on the spatial distribution of *L. maculicollis* developmental stages inevitably leads to poor targeting of the beetle with insecticides. As a result, management practices rely heavily on multiple applications of one class of insecticide (pyrethroid) over short-

mown areas as widely as possible to ensure coverage. Considering the frequent failure of this control strategy (Diaz et al. 2008; McGraw and Koppenhöfer, 2009) and weevil populations that have developed resistance to pyrethroids (Ramoutar et al. 2009), this costly and ineffective management practice should be revised.

It is known that the damage caused by *L. maculicollis* is prevalent at the periphery of the short-mown turf (Simard et al. 2007). However, there are no studies experimentally quantifying damage and documenting its spatial pattern and also no studies documenting the spatial distribution pattern of all developmental stages of the beetle at a specific site in a time series. The only previous study on the spatial distribution of the beetle was by McGraw and Koppenhöfer (2010). They contributed a novel concept on the relationship between larval distribution and host plant preference. However, as the authors argued, the low abundance of adult beetles on their study sites might have resulted in the possibility of a misinterpretation of the spatial distribution pattern. Moreover, characterizing the larval spatial distribution by one-time sampling might also have been inadequate because the larval distribution is presumably subject to the distribution of mobile adults that continuously oviposit over time.

Understanding the spatial dynamics of insects depends heavily on the ability to accurately model the spatial dynamics observed in natural situations. Spatial Analysis by Distance IndicEs (SADIE) characterizes clustering or association of count data that are spatially referenced (Perry and Dixon 2002, Park and Tollefson, 2006). In the present study, using SADIE, we aimed to characterize the spatio-temporal dynamics of developmental stages of *L. maculicollis* and the spatial distribution of the damage caused by the beetle, the host plant and various biotic/abiotic environmental factors that are assumed to influence the spatial distribution of the beetle. Also, the spatial association of these variables was examined to identify those factors that influence the insect's distribution and the expression of damage. The information presented here contributes to

our understanding of *L. maculicollis* spatial ecology and provides the foundation for improved management practices.

MATERIALS AND METHODS

Study site and design

The spatiotemporal dynamics of *L. maculicollis* abundance was monitored at the Robert Trent Jones Golf Course in Ithaca, NY (42.457723, -76.464262) over two years, 2007 and 2008. This site was selected because of a history of infestations, particularly on the fairway of hole #4, where we carried out the experiment. General maintenance practices on the golf course were carried out as described below. Between May and October, rough areas were mowed once per week, whereas fairway and intermediate rough areas were mowed three times per week. In April and November, mowing was done as needed; rough areas one to two times per month, fairways one to two times per week, the intermediate rough was not mown after mid-October. Mowing heights were 57, 32, and 12.5 mm for the rough, intermediate rough, and fairway, respectively. Fairways were fertilized in mid- to late-May with 6.1 g N/m^2, followed every 16 wk with a slow release fertilizer. Insecticides were not applied to the turf where we conducted this research. Fungicides and plant growth regulators were applied as needed. The irrigation schedule for the entire golf course was based on computer-calculated values of daily evapotranspiration from on-site weather data.

A sampling block that measured 35 m x 30 m (length x width relative to the length of the fairway) was demarcated over the fairway (mown at 1.25 cm) and included on two sides of the block areas of intermediate rough (mown at 2.5 cm, 2-m width). The sides along with the length of the fairway were in contact with rough (mown at > 5 cm), which expanded for 30-45 m away from the fairway and were in contact with tree lines. The four sides of the sampling block were

named A, B, C, and D going clockwise where B and D were the sides along the length of the fairway, and containing areas of intermediate rough (Figs. 1 & 2). The block contained 49 sampling points that were established in a Cartesian coordinate system, where (0,0) was set at the vertex of sides A and B: 2.5, 7.5, 12.5, 17.5, 22.5, 27.5, or 32.5 m x 1, 3, 9, 15, 21, 27, or 29 m. In addition, a sampling unit was defined for each point. The sampling unit was a 5 m x 2 m rectangular area whose crossing of diagonals corresponded to the sampling point and the length was parallel to that of the block.

Sampling

Sampling periods were 29 March ~ 3 November in 2007 and 17 March ~ 30 July in 2008. Adults were collected weekly from a 0.25-m^2 area demarcated by a circular template placed in the center of each sampling area. Within each template, we applied a solution of 15 ml of liquid dish detergent and 4 liters of water, which acted as an irritant to adult weevils, causing them to ascend to the top of the leaf blade where they were collected with an aspirator. The collected adults were transported to the laboratory and were identified to gender and maturation (callow or mature) through microscopic examination.

Larvae were sampled weekly by removing four soil cores (6.0 cm diameter x 6.4 cm depth; TTP1-M Turf-Tec Tubular Turf Plugger, Turf-Tec International, Tallahassee, FL) from each sampling unit. Each set of four soil cores was placed in a labeled 1-gallon plastic bag, sealed and transported to the laboratory. In the lab, a heat extraction method described by Diaz et al. (2008) was applied (32~35˚C) to extract larvae from soil cores over a 2-3 day duration. Before discarding, the dried cores were manually examined to recover any larvae remaining in plant material and thatch. All larvae were identified to instar by microscopically measuring the head capsule width as in Diaz et al. (2008).

Poa annua L. density was monitored weekly in each sampling unit until damage by *L.*

maculicollis appeared. It was measured by the point quadrat methods (Levy and Madden 1933, Robinson 1955). A 50 x 50 cm sampling frame, which was segmented into twenty-five 10 cm x 10 cm cells via 8 red elastic cords (placed at 10, 20, 30, and 40 cm in each direction creating 16 crossing points), was tossed at random within the sampling unit. The turfgrass species dominant under each of the sixteen crossing points of the red cords was identified and recorded. The percent coverage of the *P. annua* was calculated by dividing the number of points at which the plant was the dominant species by sixteen.

Visual damage of turfgrass within each sampling unit was also monitored weekly. *L. maculicollis* feeding injury causes discoloration, withering and die-off of *P. annua* turf. The dead grass was observed for the notches from insect feeding to confirm the damage is caused by the beetle. Within each sampling unit, the percent area of such damage was visually evaluated and recorded on-site. To minimize observer bias, the percentages were individually recorded for eight equally sub-divided rectangles within each sampling unit and they were summed for the entire sampling unit.

Irrigation distribution

Irrigation catch-can tests were conducted to evaluate irrigation uniformity within the sampling block at the beginning of the season in 2007 after the irrigation system was calibrated (Micker 1996 and ASAE 2000). The wind velocity was measured by a handheld anemometer, and it was less than 1 m/s during the experiment. A plastic water catch-can (tripod, 10-cm depth) was placed at each sampling point. The irrigation system was run for 10 minutes and the water depth in each cup recorded. The cycle was repeated three times. The average water depth at each sampling point and the uniformity coefficient were calculated. Since the follow-up experiment later in the season showed the consistency of the results, we assumed the irrigation uniformity was stable and the result of 2007 season was also used for 2008 season.

Thatch measurement and soil analysis

A soil core (6.0 cm diameter x 6.4 cm depth) was collected from each sampling unit at the beginning of the 2008 season, before the overwintered adults immigrated to the sampling block. The depth of thatch (a layer of un-decomposed or partially decomposed organic residues situated above the soil surface) for each core was measured from the top of the thatch layer to the soil surface. Each of 49 soil cores were individually placed in a plastic bag and transported within an hour to Cornell Nutrient Analysis Lab (Ithaca, NY) for soil fertility testing (Morgan extractable P and NO_3 (colorimetric) (kg/ha); K, Ca, Mg, Fe, Mn, Zn, and Al (Inductively Coupled Plasma Spectrometry)(kg/ha); pH (modified Mehlich); and organic matter [loss on ignition (%)], soil moisture [moisture content (%)] and soil texture.

Data analysis

Spatial patterns of *L. maculicollis* developmental stages and collected biotic/abiotic factors were analyzed using Spatial Analysis by Distance IndicEs (SADIE) (Perry 1995, Perry 1998). SADIE's cluster analysis characterizes the magnitude of aggregation of the single dataset that is spatially referenced. SADIE characterizes the spatial pattern of the target organisms by generating overall and local distance indices with their associated probabilities, and then analyzes the spatial associations of two populations by using the indices (Perry et al. 1999; Perry and Dixon, 2002). An advantage of SADIE is that it requires no assumption in the analysis of the data distribution since the results are constrained by the heterogeneity of the data set. Another advantage is that SADIE can handle zero data points, count data, binary data, semi-quantitative data, and continuous data if the data are categorized prior to analysis (Perry et al, 1999).

We employed the concept of "distance to regularity", which was interpreted in this study as the total distance the counts of *L. maculicollis* (or other observed values) at each sampling point have to move in order to achieve equal numbers at each sampling point. Each sampling

point that has a count more than the mean count of *L. maculicollis* must lose the number of insects that is equal to the difference between the observed count and mean (source) count. Similarly, each sampling point that has a count less than the mean must gain the number of insects that is equal to the difference between the observed count and mean (sink) count. Among the ways to achieve complete regularity, in which the flow of numbers is from sources to sinks, the SADIE algorithm finds the combination that has the smallest total flow distance. By summing the product of the number of insects to be moved and the shortest distances to be moved for each combination of the sampling points, the "distance to regularity", *D*, is calculated. Using a randomization procedure, *D* can be tested for statistical significance and the index of aggregation (*Ia*) is calculated by dividing *D* by expected distance to regularity. *Ia* indicates either a random, uniform, or aggregated distribution of the data when *Ia* is equal to, less than, or greater than 1, respectively (Perry et al, 1999). Indices v_i and v_j, which contribute to patches and gaps, respectively, were also calculated to show the degree of contribution of each sample to local clustering. Based on these indices, contour maps of the spatial distribution of adults, larvae, grass composition and visual damage in weekly and cumulative manner were generated by SigmaPlot (version 11.0; Systat Software, Inc., Chicago, IL, USA).

SADIE also measures the magnitude of the spatial association of two sets of spatially referenced data by overlaying cluster maps (Analysis of Spatial Association; Perry and Dixon 2002). When a patch for one data set coincides with a patch for the other, a positive association is indicated. Similarly, a negative association is indicated when a gap for one data set coincides with a gap for the other. The measure of local spatial association was represented by local spatial association indices that were calculated from local cluster indices through SADIE methodology. The overall association index (*X*), which is a correlation coefficient between the cluster indices of each set and is calculated as the mean of local association indices, indicates a random,

negative association, and positive association when X is equal to, less than, or greater than 0, respectively.

RESULTS

Population sizes were highly variable between years (Table 1), with mean peak densities within each developmental stage up to tenfold greater in the second year (adults: 19.9 and 123.8 individuals/m^2, stem boring larvae: 169.7 and 1684.4, crown feeding larvae: 366.5 and 938.8). The time to reach the peak population density also varied. For adults, peak densities in 2007 occurred in week 5, suggesting that the migration from overwintering sites lasted for at least 5 weeks and resulted in a unimodal pattern. In contrast, in 2008 the density of adults peaked twice, in week 1 and 5, suggesting that migration occurred in a bimodal manner. In each year the abundance of crown feeding larvae appeared to be directly affected by the abundance of adults that immigrated in spring, with a unimodal pattern in 2007 (peak in week 5) and bimodal in 2008 (peaks in week 5 and 8). However, among the collected stem boring larvae, first instar larvae were always present, suggesting that immigrating adults continuously laid eggs during the experimental period.

SADIE cluster indices, I_a, of cumulative counts of *L. maculicollis* developmental stages for the sampling periods, peak damage, and *P. annua* density were calculated to characterize the spatial population density patterns. Almost all I_a values were greater than 1, indicating a consistent trend toward aggregation, except for stem boring larvae in 2008 and *P. annua* in 2007 (Table 1 & Fig. 1.). The cluster indices for crown feeding larvae and damage in 2007, and adults in 2008, were statistically greater than 1 ($p < 0.05$) indicating significant aggregation. The distribution patterns were not consistent for each developmental stage, with colonization occurring both at the edge and in the middle of the experimental block. On the other hand, the

damage appeared consistently along the edge of the fairways in both years. The cluster index of *P. annua* trended toward a regular distribution (*Ia* < 1) in 2007 but an aggregated distribution in 2008; however, neither was statistically significant.

Cluster indices for abiotic factors such as thatch, irrigation uniformity, and soil properties were also calculated (Table 1). The cluster index of thatch was statistically greater than 1 ($p < 0.05$) indicating significant aggregation. Irrigation trended towards aggregation but was not statistically significant. All properties of soil texture and nutrients, except for clay contents, were significantly spatially aggregated (Fig. 2).

The results in Table 1 and Fig. 1 present the cumulative totals for 2007 and 2008. The weekly spatial distributions of *L. maculicollis* adults, stem boring larvae, crown feeding larvae, and the weekly percentage damage for each year are presented in Figs. 3 & 4. Adults migrated from overwintering sites and colonized at the edge on both sides of the fairway. In two to three weeks, they gradually moved into and distributed across the fairway. This trend was consistent over both years. Stem boring and crown feeding larvae first emerged at the edge, and then gradually across the fairway. There was a one to two week gap between the emergence of stem boring larvae and crown feeding larvae. Contrary to *L. maculicollis* spatial distributions, however, visible damage developed only along the periphery of the fairway (Figs. 3 & 4).

To assess the stability of the spatial patterns over time (the relationship between the spatial distribution pattern in one week and the following week), spatial association analysis between pairs of consecutive sampling weeks was conducted for adult, stem boring and crown feeding stages (Table 3). In 2007, the nonsignificant X values indicated that spatial distributions of adults were not stable over the weekly sampling periods, whereas the spatial distributions of adults were stable in 2008, indicated by the series of significant association values. Both stem boring and crown feeding larvae tended to maintain strong associations over time, with a

tendency for declining association towards the end of the sampling period for both years.

Spatial associations among cumulative counts of *L. maculicollis* developmental stages, percent visual damage of *P. annua*, *P. annua* density, and abiotic factors were also assessed by the SADIE association index, X (Table 4). For crown feeding larvae and damage, the spatial distributions were significantly positively associated in both years. The spatial distribution of adults and stem boring larvae were positively associated with damage but the relationships were not statistically significant. Adults and larvae did not have consistent spatial association over the two-year study. Contrary to our expectation, the *P. annua*'s spatial distribution had neither a significant nor consistent association with any of *L. maculicollis* developmental stages and damage. The spatial distribution of thatch was significantly positively associated with adults and damage in 2008. *L. maculicollis* developmental stages and damage did not have consistent associations with soil textures (clay, silt, sand) over both years. The spatial distribution of adults was statistically positively associated with most of soil chemical/physical properties that were measured in 2008 ($p<0.025$). Crown feeding larvae were significantly positively associated with soil nitrate concentration and soil organic matter content. Larvae did not show any other significant associations with soil chemical/physical properties.

Spatial association of data collected in 2007 and 2008 was also evaluated (Table 5). Cumulative densities of adults in both years had a significantly positive association, indicating that adults emerged and established in the field in a similar spatial pattern. Similarly, *P. annua* distributions in both years were also positively associated, indicating that *P. annua* remained in the same spatial area over two years. *L. maculicollis* larvae's spatial distributions were negatively associated, indicating that larval distributions had spatially opposite pattern in 2007 and 2008.

DISCUSSION

In this study we documented the spatial dynamics of the colonization of *L. maculicollis* overwintered adults and the subsequent establishment of the larval stages on a golf course fairway. This experiment was the first attempt to describe the dynamics of the spatiotemporal distributions of adult and larval stages of the beetle that were considered to be responsible for the economic damage on *P. annua* golf course turf. The results cast new light on our knowledge about the spatial structure of beetle populations on golf courses, which in the past has made it difficult to target beetle infestations in space. Because of problems with poor targeting, current management practices rely on multiple applications of pyrethroid insecticides over all short-mown areas, such as fairways, tees and greens, to suppress the density of the beetle below an economic damaging threshold.

The colonization of the overwintered adults occurred at the periphery of the fairway adjacent to the overwintering sites and gradually spread into the middle of the fairway (Figs. 3 & 4). The larval emergence also occurred at the periphery of the fairway as larvae followed the spatial distribution patterns of the adults in the same or preceding week and gradually emerged in the middle of the fairway later in the season. Despite the infestation of the adults and larvae across the fairway, the damage was observed aggregated at the periphery of the fairway and did not extend widely into the middle of the fairway, even later in the season. The prevalence of damage along the periphery of the fairway, which had long been an anecdotal observation, was clearly documented here for the first time. SADIE analysis showed further that damage was significantly positively associated with the crown feeding larvae and not with adult or stem boring stages (Table 4). This is the first experimental confirmation of a long-held belief that crown feeding larvae present at the periphery of the fairway were responsible for the damage of *P. annua.* Our results suggest that instead of the currently preferred management practice, which

is to spray insecticides against the immigrating overwintered adults multiple times over the short-mown areas, curative controls targeting crown feeding larvae, which are much less mobile than adults, along the perimeters, would be more effective and less expensive.

Because the immigration of adults to developmental sites persisted for at least 5 weeks (Table 1), a single application of insecticide is unlikely to be effective because of limited residual activity. It is preferable to suppress the overwintered adults before they lay eggs, but our data showed that beetles laid eggs soon after their arrival onto the short mown turf. In addition, because the 1st instars were always detected during the experiment, it suggests that adults continuously lay eggs from the earliest time possible until the emergence of the next generation. These phenological and reproductive characteristics lead to the inevitability of multiple applications of insecticides per season. The observed tenfold difference in the number of migrating overwintered adults between 2007 and 2008 was matched by tenfold more larvae in 2008 (Table 1), indicating a direct relationship between the number of the adults and the resulting larvae. Considering that the overwintered adults tend to aggregate at the periphery of the fairway within three weeks from the first detection, applying insecticides over the area during this period as an initial application might be a good option. This concept is also supported by the strong spatial stability that was observed at the beginning of the season (Table 3).

Since the *P. annua* density showed significant stability in spatial patterns between 2007 and 2008 (Fig. 1 and Table 5) we could assume there was no significant difference in the availability of *P. annua* to *L. maculicollis* developmental stages. However, while the cumulative density of the adult beetles maintained a consistent pattern across years, both larval stages displayed spatial instability (Fig. 1 and Table 5). These results indicate that larval spatial distribution is relatively independent from adult spatial distribution, and is subject more to the adult's oviposition patterns, which appear to be random. Here, we must consider that the damage

aggregated at the periphery of the fairway in both years (Figs. 3 & 4) and the spatial distribution of the damage was significantly positively associated with the cumulative density of the crown feeding larvae across both years (Table 4). Since the cumulative density of crown feeding larvae not only aggregated at the periphery of the fairway but also aggregated around the middle of the fairway, we can hypothesize that the crown feeding larvae emerging at the periphery of the fairway earlier in the season are more damaging than those emerging later in the season and/or that *P. annua* is more susceptible to injury by larvae at the beginning than later in the season. Although *P. annua* is adapted to a broad array of climatic conditions, they are relatively susceptible to winter injury such as dehydration and snow/ice covering for prolonged periods (Vargas & Turgeon 2004). There might be a critical period at the beginning of season in NY, while the host plant is recovering from winter dormancy, in which the host plant are likely to be more prone to insect feeding. If this is the case, it would be useful to devise a method of early protection for *P. annua* using systemic insecticides, which are incorporated by treated plants and express toxicity when the chemicals are ingested by insects, in conjunction with contact insecticides, which are toxic to insects upon direct contact.

In 2008, the spatial distribution of the damage was significantly positively associated with the distribution of thatch and sand (Table 4). Thatch is the layer of organic residue located immediately above the soil surface. It is a well-aerated, compaction-resistant medium for turf with poor nutrient and water–holding properties. In general, the turfgrasses with most of their roots confined to the thatch layer are more prone to injury from environmental stresses. Diseases and insect injury also are generally more severe where thatch is a problem (Turgeon, 2008). Sand has high porosity and poor water-holding properties. The thatch and soil with high sand are assumed to be so porous that the crown feeding larvae can easily burrow into the root zone and feed on the crown, without which the grass cannot regenerate and eventually dies off. Although

the positive relationship between thatch and sand and damage needs to be experimentally verified, cultural practices, such as removal of thatch, and/or reducing the proportion of sand by adding smaller particle soil, could greatly enhance the protection of *P. annua* by making the surrounding physical environment of the crown more compact and, thus, less accessible for the larvae. Also, how soil nutrients, that are spatially associated with *L. maculicollis* developmental stages, affect the abundance of the insect should also be investigated further.

SADIE analysis showed there were no significant spatial associations between *P. annua* and any of *L. maculicollis* developmental stages (Table 4). Our results are consistent with a less in-depth study that also did not find an effect of the host plant on the spatial distribution of either adult or larvae (McGraw and Koppenhöfer 2010). Considering that the beetle populations did not achieve a uniform distribution in our study, the traditional assumptions of host preference for short mown *P. annua* also require reconsideration. McGraw and Koppenhöfer (2010) hypothesized that mowing height is a more important factor in the beetle's selection for oviposition sites than turfgrass species, and they hypothesized that the aggregated distribution of larvae at the fairway margins was due to the adult's preference for short-mown host plants over a particular grass species. As our results show, for three weeks until moving into the middle of the fairways, the adults aggregated at the periphery (Figs. 3 & 4) which is consistent with their hypothesis. Based on the availability of the host plant, it was not necessary for the beetles to stop their dispersion because *P. annua* was uniformly distributed. One possible reason for the prolonged presence of the adults at the periphery of the fairway was the need to recover physiologically from walking the tens to hundreds of meters from the overwintering site. This speculation leads to another paradigm shift on the traditional assumption of the effect of adult feeding on the host plants. Previously adult feeding was considered to have little effect on the host plant's health (Vittum 1980). However, the assumption was based on the field observation

and there was no study that evaluated or quantified damage caused by the adults under manipulative environment. Although the adult spatial distributions were not associated with those of the damage, intensive adult feeding behavior at the periphery of the fairway during the first few weeks of the season might also contribute to the spatial distribution of the damage. Detailed studies would detect the significant decline of the host plant's growth in proportion to the increase in adult density.

The results of our study revealed the presence of complex spatial patterns and associations in *L. maculicollis* populations on the golf course. The weekly and cumulative aggregation patterns of major factors (adult, stem boring, crown feeding, damage, *P. annua*) and their spatial associations provided novel insights on the complex relationship between the beetle and various biotic/abiotic factors. The causality of the factors significantly associated with *L. maculicollis* and damage need to be tested in future studies. Understanding these relationships could lead to the development of new strategies that can accurately target the insects in space.

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Table 1. Population density (individuals per m^2) of L. maculicollis developmental stages in 2007 and 2008.

	2007			2008		
Sampling week	Adults	Stem boring	Crown feeding	Adults	Stem boring	Crown feeding
1	3.5	0.0	0.0	123.8 *	7.2	0.0
2	0.2	0.0	0.0	85.7	54.2	0.0
3	15.9	18.1	0.0	46.5	1444.3	68.6
4	15.7	146.2	3.6	21.1	1684.4 *	232.9
5	19.9 *	169.7 *	28.9	68.6 **	1664.6	436.9 *
6	16.7	135.4	108.3	57.5	1061.6	303.3
7	8.6	95.7	366.5 *	55.5	742.0	720.4
8	11.1	39.7	86.7	24.6	361.1	938.8 **
9				34.1	46.9	431.5

*: first peak, **: second peak

Table 2. SADIE cluster indices (I_a) for biotic and abiotic factors measured in 2007 and 2008

	2007	2008
	I_a	I_a
Adult	1.28	**1.82**
Stem boring	1.29	0.93
Crown feeding	**1.35**	1.02
Damage	**1.44**	1.16
P. annua	0.96	1.24
Irrigation	1.29	1.29**
Thatch		**1.68**
CLAY		1.25
SAND		**2.67**
SILT		**2.56**
Al		**2.69**
Ca		**2.67**
Fe		**1.86**
K		**1.69**
Mg		**2.26**
Nitrate		**2.18**
Organic matter		**2.21**
P		**1.46**
pH		**1.84**
Soil moisture		**2.42**
Zn		**2.21**

Statistically significant values are shown in bold ($p<0.05$)
I_a <1 indicates regular, I_a =1 indicates random, I_a>1 indicate aggregated spatial patterns.
* Data of cumulative counts of adult, stem boring, crownfeeding are used.
** Irrigatin measured in 2007 was used in 2008 due to the consistency of the result.

Table 3. SADIE association indices (X) for comparisons of *L. maculicollis* counts from each pair of consecutive sampling weeks in 2008 and 2009.

Comparison of sampling weeks	2007			2008		
	Adult	Stem boring	Crown feeding	Adult	Stem boring	Crown feeding
	X	X	X	X	X	X
1 vs. 2	-0.06			0.12	-0.05	
2 vs. 3	**0.47**			0.18	**0.52**	
3 vs. 4	0.27	**0.42**		**0.44**	**0.71**	0.20
4 vs. 5	**0.31**	0.27	**0.61**	**0.40**	**0.33**	**0.51**
5 vs. 6	0.17	**0.39**	**0.45**	**0.59**	**0.33**	**0.74**
6 vs. 7	0.28	0.10	**0.34**	**0.59**	0.31	**0.65**
7 vs. 8	**0.48**	0.18	0.07	**0.52**	0.22	0.05
8 vs. 9				**0.46**	0.29	**0.35**

Statistically significant values are shown in bold: $X > 0$ ($p<0.025$), $X<-0$ ($p>0.975$).

Table 4. SADIE association indices (X) for adult and larval stages of *L. maculicollis*, % damage of *P. annua*, and soil properties in 2007 and 2008.

	Adult		Stem boring		Crown feeding		Damage	
	2007	2008	2007	2008	2007	2008	2007	2008
	X	*X*	*X*	*X*	*X*	*X*	*X*	*X*
Adult*			-0.35	0.12	-0.11	**0.38**	0.06	0.29
Stem boring*	-0.35	0.12			0.29	**0.37**	0.28	0.31
Crown feeding*	-0.11	**0.38**	0.29	**0.37**			**0.64**	**0.67**
Damage	0.06	0.29	0.28	0.31	**0.64**	**0.67**		
P. annua	0.22	0.06	0.06	-0.06	0.24	-0.10	0.18	-0.37
Irrigation	-0.11	-0.20	0.18	-0.17	0.02	-0.19	0.07	-0.20
Thatch		**0.58**		0.18		0.25		**0.33**
Clay		0.25		0.08		-0.21		-0.17
Silt		**-0.40**		0.27		-0.20		-0.28
Sand		**0.40**		-0.16		0.29		**0.32**
Al		-0.25		0.12		-0.23		-0.15
Ca		**0.53**		-0.07		0.24		0.09
Fe		**0.46**		-0.02		-0.07		0.00
K		-0.06		0.12		0.06		-0.07
Mg		**0.50**		0.00		0.22		-0.06
Nitrate		**0.46**		0.01		**0.44**		0.33
Organic matter		**0.67**		-0.01		**0.37**		0.22
P		**0.57**		0.23		0.07		0.14
pH		-0.10		-0.31		0.00		-0.12
Soil moisture		**0.55**		-0.02		0.28		0.20
Zn		**0.63**		0.09		0.26		0.23

Statistically significant values are shown in bold: X > 0 ($p<0.025$), X<-0 ($p>0.0975$). * Data of cumulative counts of adult, stem boring, crownfeeding are used.

Table 5. Spatial association analysis of data collected between in 2007 and in 2008.

	X	p
Adult*	**0.5275**	**0.0001**
Stem boring*	-0.0984	0.7477
Crown feeder*	-0.1831	0.9009
Damage	0.1776	0.1993
Grass composition	**0.5161**	**0.0001**

Statistically significant values were shown in bold ($p<0.025$)

* Data of cumulative counts of adult, stem boring larvae, crown feeding larvae are used.

Fig. 1. Spatial distribution of cumulative density of *L. maculicollis* adults, stem boring larvae, crown feeding larvae, and the percent of damage at its peak, *P. annua* (host plant) density, irrigation, and thatch in 2007 and 2008. A patch or a gap is represented by red (vi > 1.5) or blue (vj <-1.5), respectively. *Ia* values equal to, less than, or greater than 1 indicates either a random, uniform or aggregated distribution of the data, respectively. Statistically significant values are shown in bold ($p<0.05$).

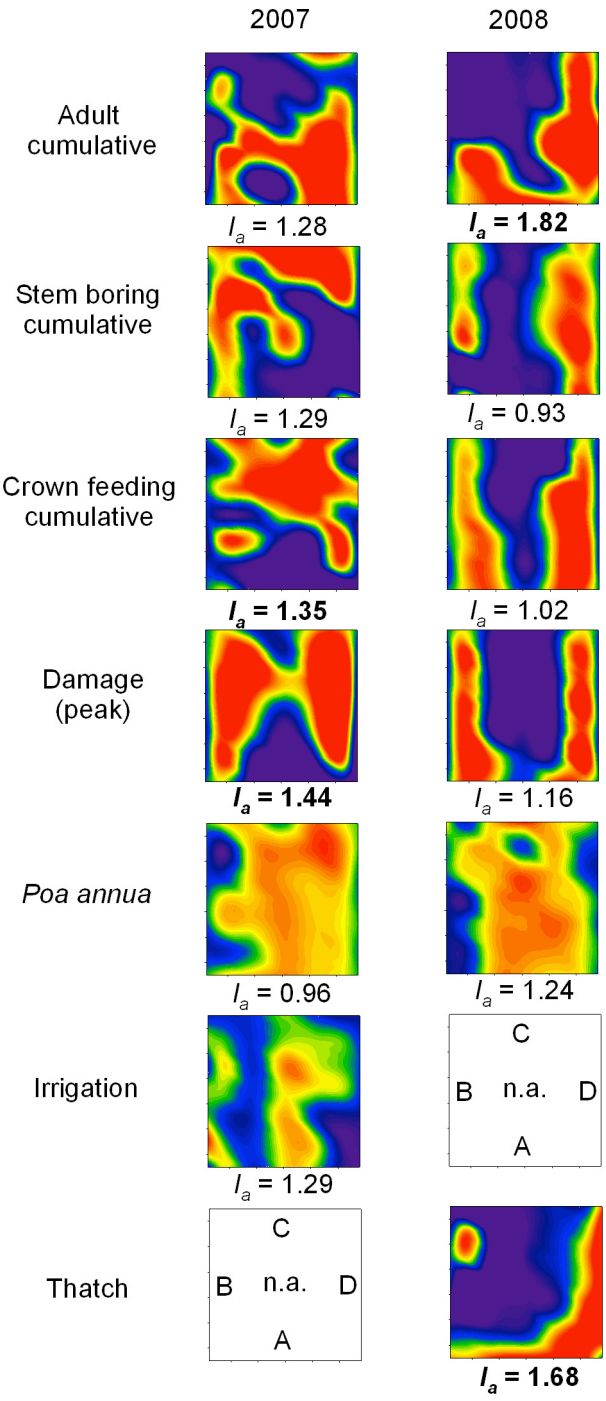

2007
2008
Adult cumulative
I_a = 1.28
I_a = 1.82
Stem boring cumulative
I_a = 1.29
I_a = 0.93
Crown feeding cumulative
I_a = 1.35
I_a = 1.02
Damage (peak)
I_a = 1.44
I_a = 1.16
Poa annua
I_a = 0.96
I_a = 1.24
Irrigation
I_a = 1.29
C
B n.a. D
A
Thatch
C
B n.a. D
A
I_a = 1.68

Fig. 2. Spatial distribution of cumulative density of soil parameters measured in 2008. A patch or a gap is represented by red (vi > 1.5) or blue (vj <-1.5), respectively. *Ia* values equal to, less than, or greater than 1 indicates either a random, uniform or aggregated distribution of the data, respectively. Statistically significant values are shown in bold ($p<0.05$).

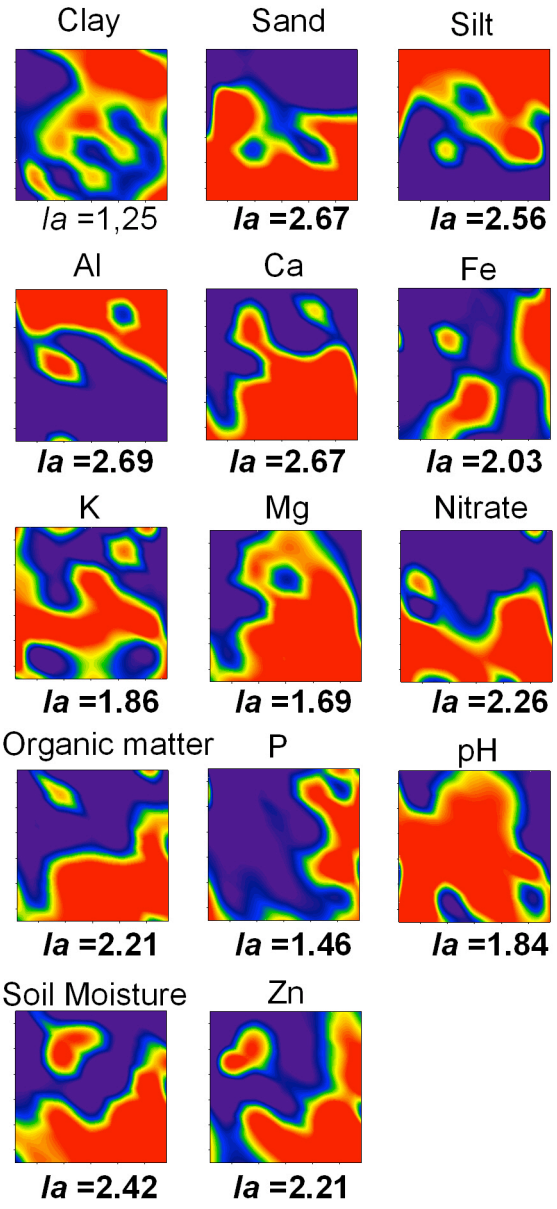

Clay
Sand
Silt
la =1,25
***la* =2.67**
***la* =2.56**
Al
Ca
Fe
***la* =2.69**
***la* =2.67**
***la* =2.03**
K
Mg
Nitrate
***la* =1.86**
***la* =1.69**
***la* =2.26**
Organic matter
P
pH
***la* =2.21**
***la* =1.46**
***la* =1.84**
Soil Moisture
Zn
***la* =2.42**
***la* =2.21**

Fig. 3. Spatial distribution of weekly density of *L. maculicollis* adults, stem boring larvae, crown feeding larvae, and the percent of damage in 2007. A patch or a gap is represented by red (vi > 1.5) or blue (vj <-1.5), respectively. *Ia* values equal to, less than, or greater than 1 indicates either a random, uniform or aggregated distribution of the data, respectively. Statistically significant values are shown in bold italic ($p<0.05$).

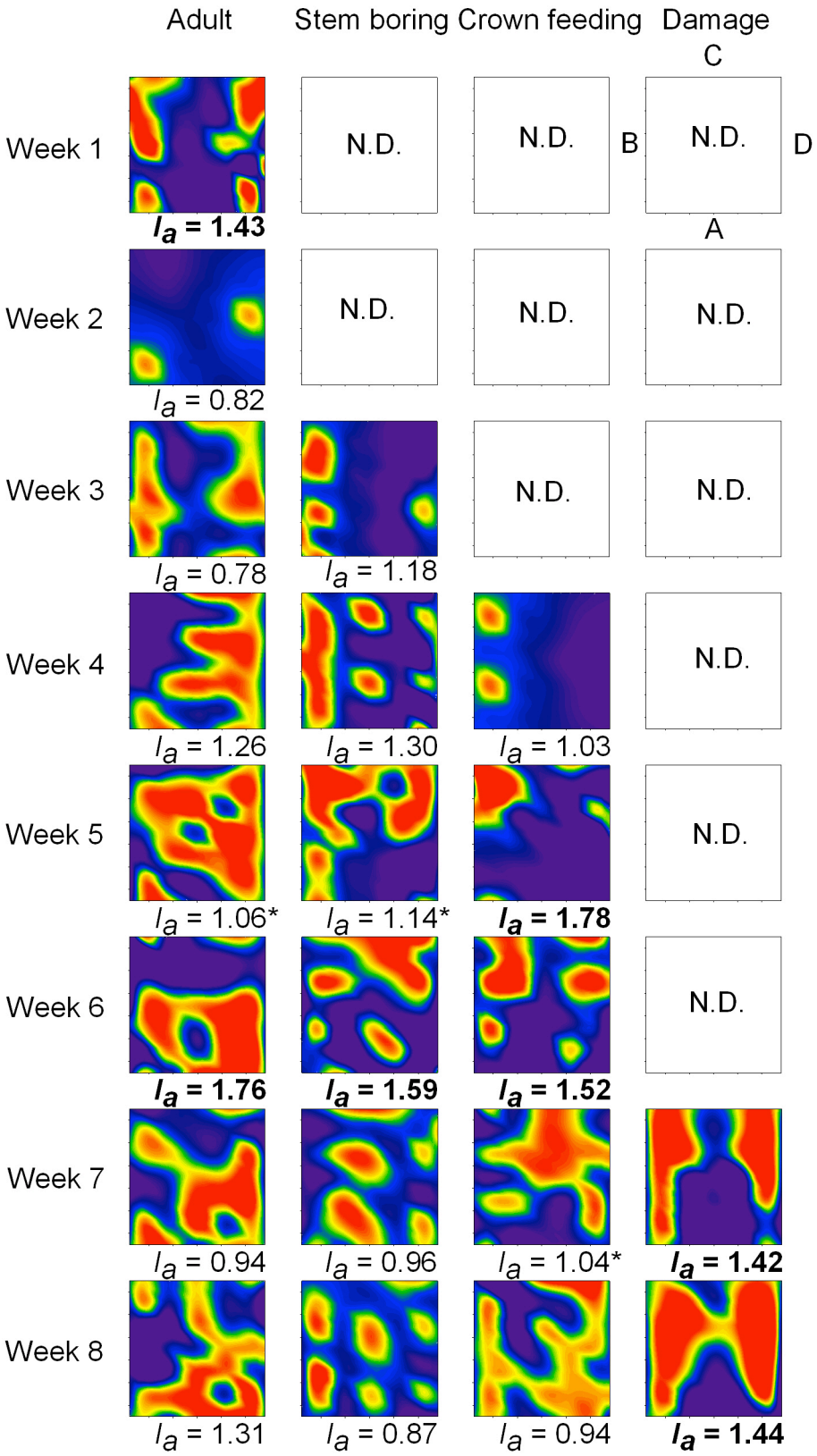
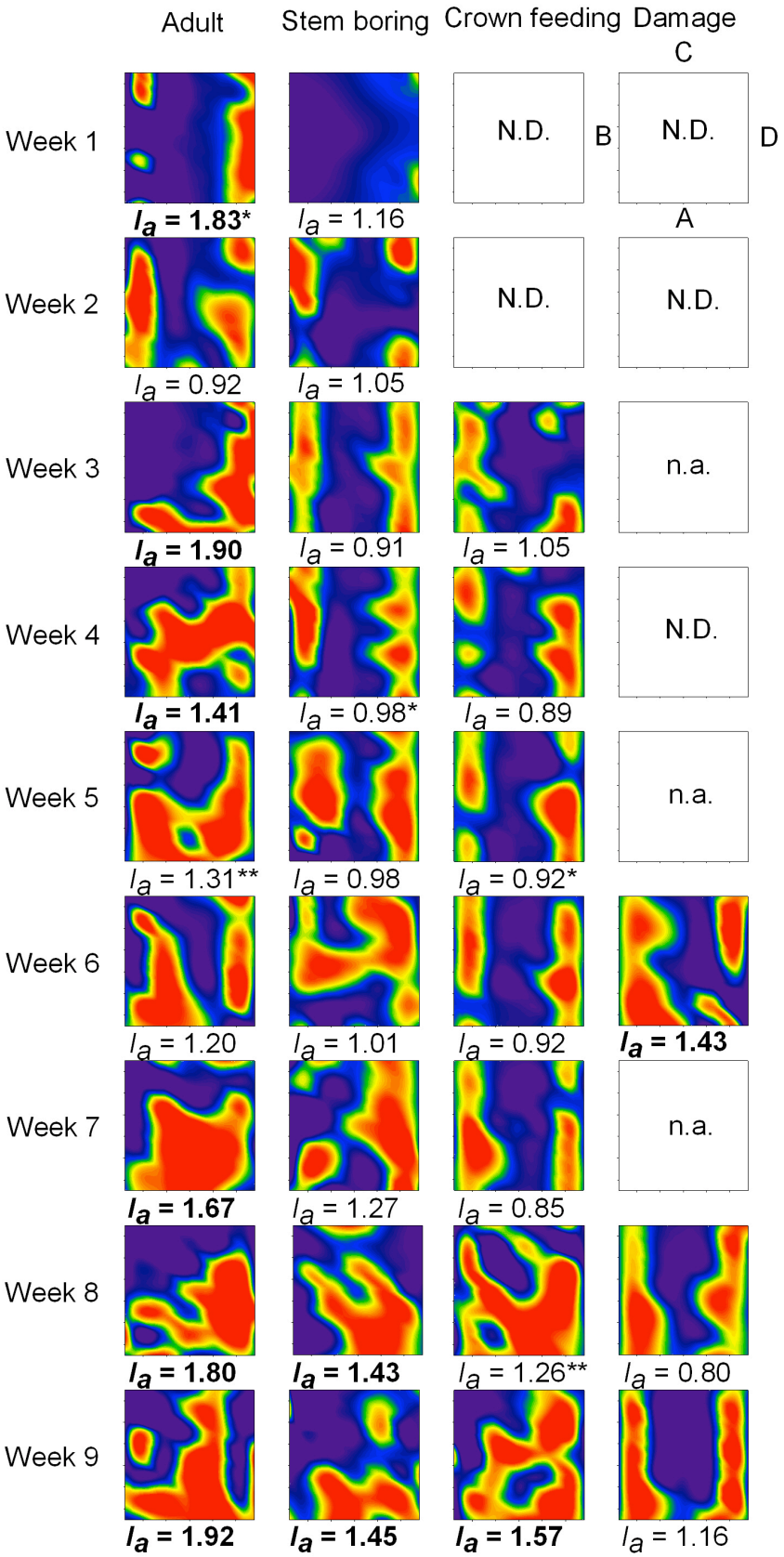

Adult
Stem boring
Crown feeding
Damage
C
Week 1
N.D.
N.D.
B
N.D.
D
I_a = 1.43
A
Week 2
N.D.
N.D.
N.D.
I_a = 0.82
Week 3
N.D.
N.D.
I_a = 0.78
I_a = 1.18
Week 4
N.D.
I_a = 1.26
I_a = 1.30
I_a = 1.03
Week 5
N.D.
I_a = 1.06*
I_a = 1.14*
I_a = 1.78
Week 6
N.D.
I_a = 1.76
I_a = 1.59
I_a = 1.52
Week 7
I_a = 0.94
I_a = 0.96
I_a = 1.04*
I_a = 1.42
Week 8
I_a = 1.31
I_a = 0.87
I_a = 0.94
I_a = 1.44

Fig. 4. Spatial distribution of weekly density of *L. maculicollis* adults, stem boring larvae (SB), crown feeding larvae (CF), and the percent of damage in 2008. A patch or a gap is represented by red (vi > 1.5) or blue (vj <-1.5), respectively. *Ia* values equal to, less than, or greater than 1 indicates either a random, uniform or aggregated distribution of the data, respectively. Statistically significant values are shown in bold italic ($p<0.05$).


Adult
Stem boring
Crown feeding
Damage
C
Week 1
N.D.
B
N.D.
D
I_a = 1.83*
I_a = 1.16
A
Week 2
N.D.
N.D.
I_a = 0.92
I_a = 1.05
Week 3
n.a.
I_a = 1.90
I_a = 0.91
I_a = 1.05
Week 4
N.D.
I_a = 1.41
I_a = 0.98*
I_a = 0.89
Week 5
n.a.
I_a = 1.31**
I_a = 0.98
I_a = 0.92*
Week 6
I_a = 1.20
I_a = 1.01
I_a = 0.92
I_a = 1.43
Week 7
n.a.
I_a = 1.67
I_a = 1.27
I_a = 0.85
Week 8
I_a = 1.80
I_a = 1.43
I_a = 1.26**
I_a = 0.80
Week 9
I_a = 1.92
I_a = 1.45
I_a = 1.57
I_a = 1.16

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CHAPTER FOUR

OVIPOSITION BEHAVIOR, DEVELOPMENT AND REARING OF *LISTRONOTUS MACULICOLLIS* KIRBY (COLEOPTERA: CURCULIONIDAE)

ABSTRACT

Listronotus maculicollis Kirby (Coleoptera: Curculionidae) adults were tested for seasonal changes in oviposition behavior and longevity. Overwintered adults collected from overwintering sites, before the spring immigration, initiated oviposition within five days from the collection under laboratory condition. The overwintered adults oviposited a similar average number of eggs throughout the entire collection period between April and June. In contrast, the first generation adults, which normally emerge in late June, rarely laid eggs in July. To study in greater detail aspects of oviposition, development, and longevity, a wheat germ-based artificial diet was developed and compared with lettuce. The complete development, from egg to adult, took 90 ± 4 days (± standard errors) on the diet, compared with 67 ± 4 days on lettuce. Mortality during development was also lower on lettuce relative to the diet. The median adult longevity while feeding on wheat germ-based diet was 471 days for female and 200 days for male. These results, taken together, suggest the possibility that overwintered adults survive throughout the season and that they are a significant source of the larvae emerging in mid-summer, raising questions about our current perspective on voltinism for this species. Our findings provide new information on the oviposition behavior and development of *L. maculicollis* and could contribute to the development of a novel management strategy that is biologically and ecologically based.

INTRODUCTION

Listronotus maculicollis Kirby (Coleoptera : Curculionidae) is a serious insect pest of

Poa annua L. (Poales: Poaceae) turf on golf courses in the Northeastern U.S. (Potter 1998, Vittum et al. 1999). The beetle was first detected damaging turf in Connecticut in 1931 (Britton 1932), and infestations are now experienced across the Northeast and into the Mid-Atlantic, including north to Maine and Quebec, west to Ohio, south to North Carolina and Georgia (Tashiro 1976, Watschke et al. 1994; Potter, 1998; Vittum et al. 1999; McDonald and Dernoden, 2005; Simard et al. 2007; Koenig et al. 2008; O'brien and Hartwiger, 2011).

Historically, the primary control strategy has been insecticide applications against the overwintered adults, which disperse from overwintering sites (edge of woods, wind breaks, etc.) to short-mown turf (fairways, greens, tees) to reproduce in spring (Diaz and Peck 2007; Cowles et al. 2008; Vittum et al. 1999). In an attempt to eradicate the overwintered adults, multiple insecticide applications against a single generation are commonly observed. As a result of the multiple applications and the overwhelming reliance on pyrethroid insecticides in the recent years, resistance among certain populations of *L. maculicollis* has emerged (Ramoutar et al. 2009). The difficulty in timing of chemical control arises from the beetle's complex seasonal behavior. *L. maculicollis* overwinters in various microenvironments largely along the edge of defined tree lines (Diaz and Peck 2007). During the spring, the beetle walks from overwintering sites to the short-mown turf area and this activity can persist for more than six weeks (Diaz and Peck 2007). The emergence of larval stages are observed soon after the arrival of the adults and because of the lengthy dispersal period the beetle populations exhibit an asynchronous age distribution, where all developmental stages except for pupae are observed at any given time (Diaz et al 2008). However, it also is known that the timing of the emergence of adults of the summer generation is generally synchronized (Vittum et al. 1999; Diaz et al 2008). Based on these facts, one possible explanation why a single chemical application is often unsuccessful in suppressing population levels below damage thresholds would be that the overwintered adults

become reproductively active in a short period of time after overwintering, and are ovipositing over a relatively long period during the spring. If true, this also implies that larval development rates must vary greatly so that the emergence of adults of the next generation becomes relatively synchronous. Although it is important to test hypotheses about these life-history traits, basic information about its reproductive behavior is not available and lack of laboratory rearing method for *L. maculicollis* has hampered progress studying basic biological characteristics such as developmental rates and long-term longevity.

In this study, oviposition behavior of field-collected *L. maculicollis* adults was studied with emphasis on fecundity. Also, to develop a laboratory mass-rearing protocol, different ingredients for inclusion in an artificial diet were screened, and different vegetables as food and oviposition substrates were tested. Development and longevity of insects were investigated using both artificial diet and the vegetable diet in order to better understand phenology of *L. maculicollis* and modify current management strategies accordingly.

MATERIALS AND METHODS

Insect collection and incubation method

All insects were collected from Robert Trent Jones Golf Course, Ithaca (42.4577, -76.4643) unless indicated otherwise below. Adults were collected from a plastic template demarcating 0.25-m^2 circles placed on turf. As an irritant, a solution of 15 ml of liquid dish detergent in 4 liters of water was applied within each template. After 2-5 min, adults that ascended to the top of the grass blades were aspirated into a 100-ml centrifuge tube containing a sheet of paper towel to absorb the remaining soap solution from the surface of the insects. The collected adults were transported to the laboratory and were identified to gender and maturation (callow or mature) through microscopic examination. This method was used for each of the

following experiments. The collection method was repeated until the target number was obtained.

In the following experiments, males and females from the pool of adults collected on the same day were randomly selected and paired. All the containers used for incubating insects were kept in a growth chamber at 20 ˚C, 80% relative humidity under 16:8 h light:dark cycle. All petri dishes had ten 1 mm diameter ventilation holes and were sealed with Parafilm around the circumference during incubation. In the trial using plants as oviposition substrates, the plants were replaced every day since we examined them destructively.

Statistical analysis and graphing were conducted using R, version 2.14.0 (R Development Core Team 2011).

Regional variation in oviposition

In previous population studies at these two sites, the density in Ithaca was consistently higher (~ 8 times) than that in Fayetteville (Diaz et al. 2008) and differences in fecundity were suspected as at least part of the reason. To study if different populations have a significant difference in fecundity, overwintered *L. maculicollis* adults were collected from the fairways of two golf courses in NY in 2006 to assess population variation in oviposition: Robert Trent Jones Golf Course, Ithaca and Onondaga Golf and Country Club, Fayetteville (43.0283, -76.0409). The collections were made on June 6, 13, and 22 in Ithaca and on June 5, 12, and 19 in Fayetteville.

Each of ten pairs was placed in a 100 mm x 15 mm plastic petri dish with a whole *Poa annua* L. planting whose root was wrapped with a piece of moist paper towel. The plant was collected from the same field, and on the same day, from which the insects were collected. The plants were separated from the soil and thoroughly washed in water. After trimming tillers, the plants were examined under the microscope and those free of eggs were selected. The petri dish and the plant inside were examined daily for 7 days, recording the number of the eggs.

Days required to initiate oviposition in spring

Overwintering *L maculicollis* adults were collected from the overwintering sites, the woods adjacent to the fairways where the infestation of *L maculicollis* was observed in the previous years, on February 18, March 15, and April1 in 2008. The leaf litter and the top 1cm of soil were removed from each sampling area and placed in a plastic bag. All the bags were immediately transported to the laboratory. The leaf litter collections were examined for overwintering adults, which were moved to a plastic cup. The soil portion of the sample was immersed into lukewarm water (30 – 35 ˚C) and the overwintering adults that ascended to the surface were collected. The collected adults were identified to gender and maturation (callow or mature) through microscopic examination.

Each of thirty male/female pairs was placed in a 100 mm x 15 mm plastic petri dish with a whole *Poa annua* L. as described above. The petri dish and the plant inside were examined daily and the first date to detect eggs was recorded.

Oviposition of overwintered adults and first generation adults using *P. annua*

The collection for the overwintered adults was done on April 22, May 20 and June 11 in 2008; the adults of the first generation were collected on July 1, July 8, July 15 and July 22 in 2008.

Each of thirty male/female pairs was placed in a 100 ml centrifuge tube that contained a whole *Poa annua* L. after processing as described above. Each tube was capped with lid that had 10 ventilation holes (1 mm diameter each) and was incubated. After 5 days of incubation, the inside walls of the tubes and the plants were thoroughly examined and the number of eggs laid were recorded. The experiment was run on the day of each collection, using the adults collected on the same day. Thus, there were seven individual replicates of the experiment, with 10 pair of beetles per trial.

Oviposition of overwintered adults and first generation adults using lettuce

L. maculicollis adults were collected from the fairway in Robert Trent Jones Golf Course, Ithaca, NY. The collection of overwintered adults was done in May and the collection of the first generation was done in July in 2009.

Each of thirty male/female pairs was placed in a 100 mm x 15 mm petri dish that contained a filter paper (45 mm diameter, moist with 1 ml of water) and 20 mm square of chopped vein of organic romaine lettuce purchased from a local grocery store. For the females collected in July, After 5 days of incubation, the inside wall of the petri dish, the filter paper and the lettuce were thoroughly examined and the number of eggs was recorded.

Development & longevity using artificial diet

Source of insects

The overwintered adults were collected during April and May, 2007. To screen out unhealthy individuals, each adult was placed in a 30 ml plastic cup and fed on *Poa annua* L clippings for 7 days. Any individuals that died were discarded. From the screened individuals, thirty male/female pairs were randomly selected and each pair was placed in a 100 mm x 15 mm plastic petri dish with a whole *P. annua* as described above. The petri dish and the plant inside were examined after 5 days, and the eggs found were recovered and kept on filter paper (45 mm diameter) with 1ml of water that was placed at the bottom of 50 mm x 10 mm Petri dish.

Artificial diet

To study in better detail the developmental rate and longevity of *L. maculicollis*, the suitability of the following artificial diets were tested: 1) agar-based diet with *P. annua* powder (agar (5 g), dried *P. annua* powder (40 g), ascorbic acid (1 g), Methyl-p-hydroxybenzoate (0.5 g), sorbic acid (0.5 g), USDA vitamin premix (Bio-Serve, Frenchtown, NJ, #6265; 1 g), formaldehyde 10% (0.5 ml), distilled water (300 ml)); 2) paste of *P. annua* clippings (fresh-cut

P.annua clippings from greenhouse were ground in a mortar); 3) a wheat-germ based lepidopteran diet (Chatterji et al. 1968); 4) a wheat-germ based weevil diet (Malone & Wigley 1990). 10 ml of each diet was placed in a 30-ml plastic shot cup. Ten male/female pairs of field-collected adults were individually placed in each diet and were incubated for thirty days. Thirty laboratory-laid eggs were individually placed in each diet and were incubated for sixty days. The criteria used were mortality and feeding activity of the adults and larvae of *L. maculicollis*.

In a separate, but related experiment, we used an artificial diet modified from one developed for the argentine stem weevil, *Listronotus bonariensis,* (Malone & Wigley 1990). The following ingredients were mixed and autoclaved at 121 ˚C for 15 minutes: distilled water (1000 ml), agar (40 g), casein (30 g), cellulose powder (100 g), Wesson's salts (5 g), wheat germ (30 g), sucrose (30 g), glucose (3 g), trehalose (3 g), methyl paraben (1250 mg), and sorbic acid (900 mg). After autoclaving, cholesterol (500 mg) and linseed oil (2 ml) were immediately added. After cooling the diet mix to 70˚C, USDA vitamin premix (Bio-Serve, Frenchtown, NJ, #6265; 6 g) was added and mixed thoroughly. The diet was poured into plastic Petri dishes and left to set.

Study on development

The *L. maculicollis* diet was cut out into cylindrical plugs (20 mm diameter x 10 mm). Each plug was placed in a plastic cup (30 ml), and a 2 mm deep slit (5 mm long) was cut by a knife sterilized with 98% ethanol. A laboratory-laid egg from field-collected adults in 2007 (as shown above) was transferred with a fine paintbrush to a cup and placed between the slit. The survival of the larvae was checked every day until the development was complete. If an exuvia was observed, it was recorded so as to track the developmental rate of each stage. A set of 30 cups was prepared for each of three replicates.

Study on longevity

Thirty male/female pairs of adults collected in May 2007 were placed in a 100 mm x 15

mm Petri dish with a block of *the L. maculicollis* diet (20 mm x 20 mm x 10 mm). The insects were transferred to a new Petri dish with new diet every week and their survival was recorded.

Development and fecundity of lab-reared adults using various plants as diet

Beans, carrots, celery, romaine lettuce leaf, romaine lettuce vein, and baby spinach were evaluated as diet for rearing both adults and larvae of *L. maculicollis*. The vegetables were USDA organic and were purchased from a local supermarket. Adults fed on all of the above but only laid eggs into lettuce leaf, lettuce vein and spinach; only lettuce vein was suitable for longer term studies for practical reasons. Larvae could burrow into and feed on these three, however; lettuce leaf decayed very quickly and the larvae were difficult to observe in spinach. Thus, lettuce vein was selected as a potential diet substrate.

Overwintered adults were collected in April 2009. Each of thirty male/female pairs was placed in a 100 mm x 15 mm petri dish that contained filter paper (45 mm diameter, moist with 1 ml of water) containing a 20 mm square of chopped lettuce vein of organic romaine lettuce. After 5 days of incubation, the inside wall of the petri dish, the filter paper and the lettuce were thoroughly examined and all the eggs were recovered.

A 20 mm square of chopped vein of organic romaine lettuce was placed in a 50 mm x 15 mm Petri dish. Ten slits (2 mm depth x 5 mm long) were made by a sterilized knife on the lettuce and one egg was placed in each of them. After larvae emerged, each of them was separated to individual Petri dishes with new lettuce. The survival of the larvae was checked daily until development was complete. If exuviae were observed, they were recorded so as to track the developmental rate of each stage. The larvae were transferred to new Petri dishes with new lettuce every seven days. The development of a total of 90 individuals was studied.

RESULTS

Results from the series of trials on oviposition, development and longevity of *L. maculicollis* are organized with respect to estimates of fecundity, timing of oviposition, development and longevity.

Fecundity.

There was no statistical difference in the number of eggs obtained from 10 pairs of adult *L. maculiocollis* collected from two geographically distinct (70 km apart) golf courses (Table 1). At both sites, the number of eggs declined as time passed, as was also observed for adult abundance and emergence of stem boring larvae in the field in that year (data not shown).

Oviposition.

For the overwintered adults collected in February, March and April of 2008, sampling date was found to have a significant impact on the number of days to initiate oviposition (Fig. 1, $F_{2,87}$=6.69, P<0.01). It took 4.2 ± 1.5 (± standard error) days and 4.4 ± 1.7 days for the adults collected in February and March; they were not significantly different from each other (t_{58}=0.377, P = 0.92, TukeyHSD). It took significantly shorter time, 2.6 ± 1.2 days, for the overwintered adults collected in April to initiate oviposition than the adults collected in February and March (t_{58}=-2.96, P = 0.01; t_{58} = -3.33, P <0.01, respectively).

There was no statistical difference in the mean numbers of eggs oviposited on *P. annua* by pairs of adults collected in April, May and June of 2008 (4.1 ± 2.3, 3.5 ± 3.5, and 3.5 ± 2.3, respectively; $F_{2,87}$ = 0.26, P = 0.77, Fig. 2.). However, the adults collected in July of 2008 rarely laid eggs over four consecutive weeks; the mean number of eggs ranging from 0.0 to 0.5 (Table 2). These results were consistent with the results for adults collected in April or July in 2009 and provided a chopped vein of lettuce for oviposition (Table 3). Pairs of overwintered adults collected in April laid 20.1 ± 4.4 eggs, with the maximum number of 84. On the other hand, the first generation adults collected in July laid 0.3 ± 0.2 eggs, with the maximum number of 4 (Figs.

3&4).

Development and Longevity

Adults were observed to feed on all the diets initially tested (agar-based diet with *P. annua* powder, paste of *P. annua* clipping, wheat-germ based lepidopteran diet, and wheat-germ based weevil diet), but laid no eggs. Only a total of four individuals died during the experiment. Only larvae reared on the wheat-germ based weevil diet completed the development to adults. Other diets were antagonistic to the proper development especially for early instars, which were trapped by surface tension on agar-based diet, did not feed on grass-clipping pastes, and could not burrow into wheat-germ based lepidopteran diets. In contrast, the larvae could burrow into wheat-germ based weevil diet, fed, grew, and pupate in the diet.

There were only nine of ninety individuals that successfully completed development on the *L. maculicollis* artificial diet as a single food source (Table 4). The mortalities of trials one to three were 90 %, 100 %, 80 %, respectively. On average, they took about 90 days to develop from egg to adult. All nine individuals pupated in cells that they made inside the artificial diet. The mortality of the larvae on the artificial diet was extremely high and >85 % of death occurred when the larvae were first and second instars. Once the larvae become the third instar, the mortality dropped significantly. For the ninety individuals reared on lettuce, twenty-four (ten females and fourteen males) completed development from egg to adult (Fig. 5). The average developmental time was 68.0 ± 6.7 days for females and 66.1 ± 6.1 days for males. The overall mortality was 73%. Exuvia were not recovered from all the larval stages, and thus, the development rate for each stage could not be determined. All twenty-four individuals pupated outside of the lettuce vein. Out of twenty-four individuals successfully reared to adult on lettuce, ten randomly generated pairs were incubated in a petri dish with lettuce veins. None of the pairs laid eggs until 264 days after the first pairing. When the first eggs were laid, only three original

pairs remained. All of them laid eggs within a week from the first observed oviposition. The mean total number of eggs collected from the first ovipositions was 5 ± 1.2 eggs.

The survivorship curves of sixty overwintered adults maintained on artificial diet (thirty males and thirty females) indicate that females survived longer than males during the experiment ($X^2 = 12.6$, df = 1, $P < 0.01$, Mantel-Haenzel test, Socal & Rohlf, 1995; Fig. 6). The median longevity of females was 471 days, whereas that of males was 200 days. The maximum longevity observed was 965 days for females and 673 days for males.

DISCUSSION

This study documented characteristics of reproductive behavior and longevity of field-collected *L. maculicollis* under laboratory conditions, and examined the complete developmental time of laboratory-reared *L. maculicollis*. In addition, rearing methods with raw vegetable substrates (Romaine lettuce vein) were also developed and showed a certain degree of practicality for future experiments. The artificial diet was suitable for maintaining adults but not for the development of larval stages.

The phenology and life cycle of *L. maculicollis* can differ substantially within its geographical distribution and between years (McGraw and Koppenhöfer 2007). This variation was originally considered to be due to the differences in climate pattern, but in another study by Diez et al. (2008), large differences in population abundance (~8 times) were observed in similar climate areas in the same years, over a three-year period. Consequently, we expected large differences in fecundity of the different populations that inhabit similar climate areas. Surprisingly, there was no significant difference between two populations that were collected from two geographically distinct (70 km apart) golf courses (Table 1). The consistency was observed not only in the number of eggs but also the pattern of the declining fecundity toward

the end of the generation defined by population fluctuation curves. Under laboratory conditions, it seems there was no difference in average reproductive performance between the pairs collected within the same week at the different sites. Thus, other environmental conditions such as availability and nutritional states of host plants, soil types, etc. seem to influence the reproductive performance and abundance of the beetle in the field.

L. maculicollis is considered to undergo a state of reproductive diapause that is similar to the one documented for in *L. bonariensis* (Goldson 1981, Rothwell 2003). Our study showed the insect could terminate the reproductive diapause within 5 days of exposure to 20 ˚C (Fig. 1). The termination of reproductive diapause seemed to depend on the temperature and availability of food. It is reasonable to consider that the overwintered adults encounter host plants while they walk tens of meters toward the short-mown turf area from the overwintering sites and they are reproductively active by the time they get to the developmental site. If this were the case, targeting the overwintered adults on the fairway to suppress the occurrence of the following generation would likely be ineffective, because, by the time of the treatment, the overwintered adults that arrived earlier have already laid many eggs and a portion of them will have already hatched. These eggs and larvae are protected inside of the grass and insecticides will not be efficacious. In this sense, it would be better to target the overwintered adults before they get to the fairway.

Asynchronous generations are often observed in *L. maculicollis* populations (Vittum and Tashiro 1987, Vittum 1995, Diaz et al 2008). A continuous and prolonged oviposition period was expected to be a contributing factor. The field-collected adults in April, May and June, when only overwintered were present, showed similar fecundity throughout the period (Figs. 2 & 3, Table 3). Thus, the overwintered adults continuously laid eggs after the arrival onto the oviposition sites. In contrast, adults collected throughout July rarely laid eggs (Tables 2 & 3, Fig.

4). This phenomenon is explained by the emergence of the adults of the first generation, which are not sexually mature. This explanation is supported by Rothwell's study (2003) that showed a sharp decline of the number of eggs, the rate of insemination, and the size of reproductive organs observed in the dissection of adults collected in the month of July. However, this does not explain the rise of larval populations that are observed in mid-June through July after the emergence of the adults of the first generation. Diaz and Peck (2007) showed that the spring immigration of adults ceased by the mid-June and another immigration of the adults was detected in July in different experimental sites. The adults immigrating later might be the overwintered adults, because the directional pitfall traps did not detect emigration of adults from the fairway prior to the second immigration (Diaz and Peck 2007). Although the latter immigration was smaller than the former, this second immigration of the overwintered adults could be the main source of the appearance of the larvae in July. Rothwell's study (2003) showed a sharp increase in reproductive capacity of field-collected adults in August. If this is the case, what we currently call the "second" generation might be actually the first generation. Although there is no definitive evidence either way, our results and the results of Rothwell indicate more research is necessary to understand voltinism and phenology of this insect.

The developmental time to the adult stage and adult longevity of *L. maculicollis* were tested on a new artificial diet. The diet was suitable for adults in the lab but not practical enough to use as a mass rearing food substrate. The days for completion of development, which was assumed to be 60 days based on a previous field study (Diaz et al. 2008), took about 90 days (Table 4). The delay in the development may indicate that nutrients that are critical for larval growth are scarce in the diet, or the concentration of some ingredients is high enough to be toxic. Since mortality dropped significantly after the larvae became third instars, the imbalance of nutrients seemed more crucial for the first and second instars. Moreover, the physical structure of

diet seemed unfavorable for the early instars because they could not efficiently burrow into the diet. Many early instars were often found roaming on the diet and looking for a space to burrow into. Even though the diet was prepared with a slit, many larvae chose not to go between the slits and died. There might be a physical structure that strongly affects the larval burrowing activity, such as a layered tissue structure as grass shoots possess (Turgeon 2008).

The median longevity of the overwintered females and males reared on the artificial diet (471 and 200 days) indicated the longevity of the beetle on the field could be long enough to survive through a season. This might be another explanation for the asynchronous population dynamics observed in the field, the long-term spring immigration, and the possible summer immigration. Although the insects have the potential to survive through a season, there is ordinarily a decline of adult populations on golf course fairways right before adults of the first generation appear. Since no emigration of the adults from the fairways was observed in the directional pitfall traps (Diaz and Peck 2007), the death of the overwintered adults on the fairway could be a response to certain environmental changes, such as photo period, degree-day accumulation, physiological changes in host plants, etc. For another explanation, Diaz and Peck (2007) proposed such that the emigration was mainly achieved by flying thus the directional pitfall trap on the ground could not detect the insect movement. To investigate movement and population size of overwintered adults in summer, mark-recapture method (Williams et al. 2002) is a viable option for future studies.

There was no significant difference in the developmental rate between females and males on lettuce vein and the average developmental time (female: 68.0 ± 21.1, male: 66.1 ± 22.9) was close to theoretical time (ca. 60 days) assumed from the previous population (demographic) study (Diaz et al. 2008). A comparison of the nutritional value of the lettuce vein and the artificial diet might reveal what nutrients are critical for the beetle's growth. The physical

structure of the lettuce vein seemed to be favored by all stages of larvae since larvae roaming outside of the lettuce were rarely observed. Time to complete development was normally distributed with a large variance (Fig. 5). The shortest developmental time observed was 25 days and the longest was 113 days. It is unknown what induced this vast difference in the developmental rate given individuals reared under the same laboratory conditions. If this variation also occurs in the field, this is another explanation of the asynchronous generations we have observed. It took 264 days to lay their first eggs onto lettuce for the lab-reared adults that were reared on lettuce from the eggs of field-collected overwintered adults. If they had remained in the field, they would have had emerged as adults sometime in July. 264 days later, which is about 8.5 months, corresponds to the spring immigration period of the following year. Rothwell (2003) showed the size of reproductive organ of the field-collected adults, presumably a mixture of overwintered adults and first generation adults, sharply declined after August and remained very small until February when the size gradually grew larger even in the overwintering sites. This indicates *P. annua* is not an essential element for reproductive maturity because the plant is rarely observed in overwintering sites. This corresponds with the observation that the time to the first oviposition of lab-reared adults was not stimulated by supplement of *P. annua* as a food/oviposition substrate (data not shown). The lab-reared adults were kept at constant temperature and day length; they did not experience any fluctuation of them as the field population would. The seasonal change of reproductive organs might happen regardless of the temperature and day length.

Overall, this study provided new information on the life history, especially on oviposition behavior and development, of *L. maculicollis*. It implies the necessity of modifying the conventional views of the seasonal changes of *L. maculicollis* development that were largely based on anecdotal observation. To better understand the beetle's life history, it would be helpful

to investigate the variations discussed in this study in a larger geographical context. Such detailed studies on the basic biology of this insect could contribute to the development of a novel management strategy that is biologically and ecologically based.

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Table 1. T-test results for mean number of eggs obtained from 10 pairs of *L. maculicollis* adults collected at two different golf courses in New York for three consecutive weeks in June 2006. (SE = standard error)

	Fayetteville	Ithaca		
	n=10 (pairs)	n=10 (pairs)		
Sampling week	Mean ± SE (No. of eggs)	Mean ± SE (No. of eggs)	t_{18}	p
week 1	4.5 (± 2.3)	5.5 (± 3.8)	0.34	0.37
week 2	2.6 (± 1.6)	3.1 (± 1.8)	0.30	0.38
week 3	1.1 (± 0.9)	2.0 (± 1.2)	0.31	0.19

Table 2. Sum and mean of the number of eggs laid by pairs or females of *L. maculicollis* collected from the field for four consecutive weeks in July 2008. Pair is a pair of male and female of *L. maculicollis* randomly selected from the pool of the field-collected insects and Single is arandomly selected female.

	Pair			Single		
	n=30			n=30		
Date	Sum	Mean	SE	Sum	Mean	SE
July 1	1	0.0	0.0	0	0.0	0.0
July 8	0	0.0	0.0	1	0.0	0.0
July 15	6	0.2	0.2	15	0.5	0.5
July 22	0	0.0	0.0	7	0.2	0.1

Table 3. Number of eggs oviposited onto lettuce by 30 pairs of *L. maculicollis* adults collected in April and July 2009.

Stage	n	Sum of eggs	Mean	SE	Max	Min
OWA	30	602	20.1	4.4	84	0
1st generation	30	10	0.3	0.2	4	0

Table 4. The number of *L. maculicollis* that completed its development from eggs to adults on the artificial diet and mean number of days to complete the development. Eggs were laid in the laboratory by overwintered adults collected in April and May 2007.

Trial	n	Complete development	Mortality	Mean development days ± SE
1	30	3	90%	86.7 ± 4.3
2	30	0	100%	-
3	30	6	80%	90.0 ± 3.4

Fig. 1. Mean number of days (± SE) taken for overwintering *L. maculicollis* adults to initiate oviposition. The adults were collected from overwintering sites in February, March and April of 2008. Different letters above bars indicate significant differences ($P<0.05$) between boxes tested with Anova followed by post-hoc Tukey's HSD test.

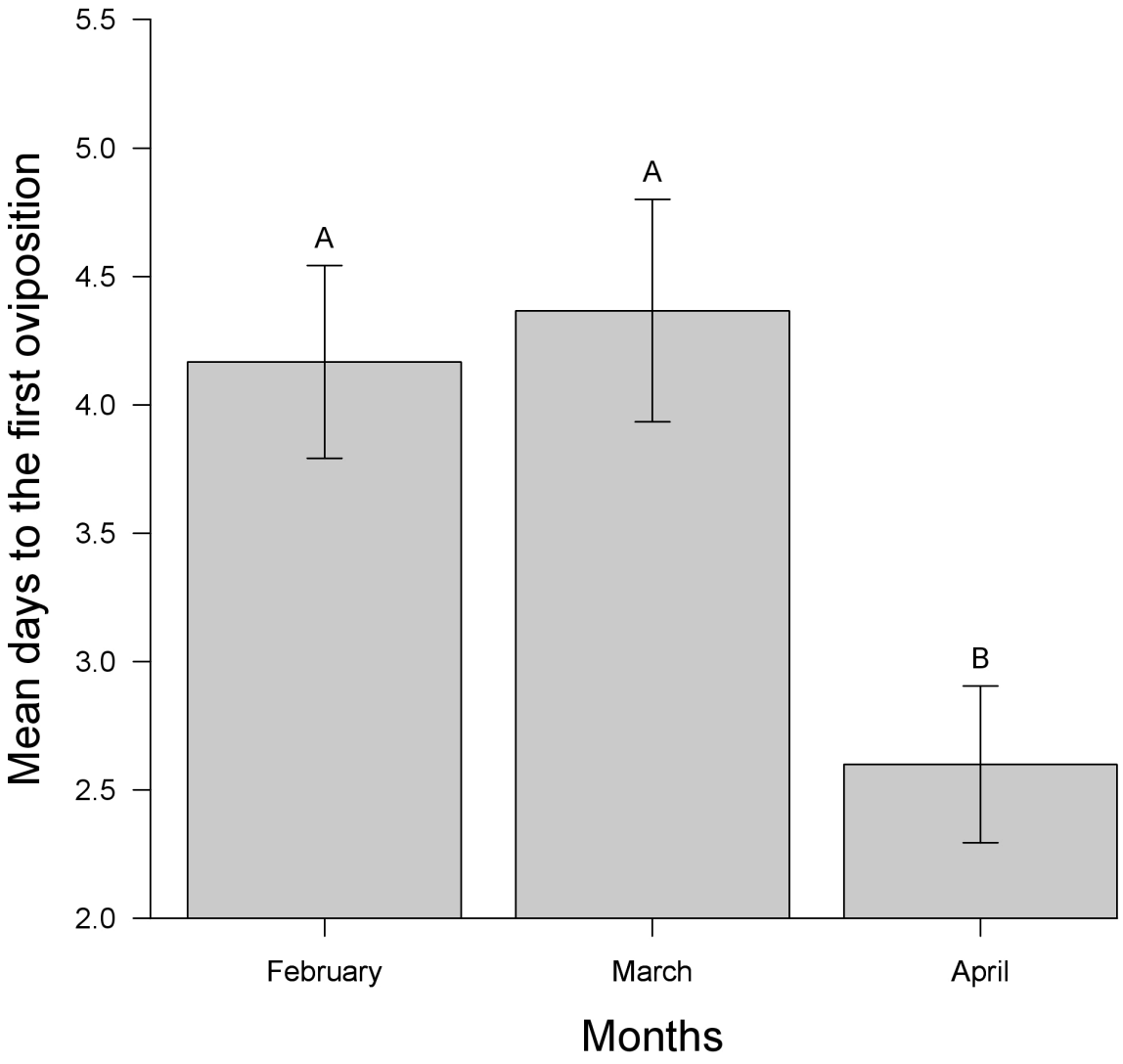

Mean days to the first oviposition
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
A
A
B
February
March
April
Months

Fig. 2. Mean number of eggs (± SE) oviposited by a pair of *L. maculicollis* collected from a golf course fairway in April, May and June of 2008. Different letters above bars indicate significant differences ($P<0.05$) between boxes tested with one-way Anova. ($F_{2.87} = 0.2628$, p-value=0.7695).

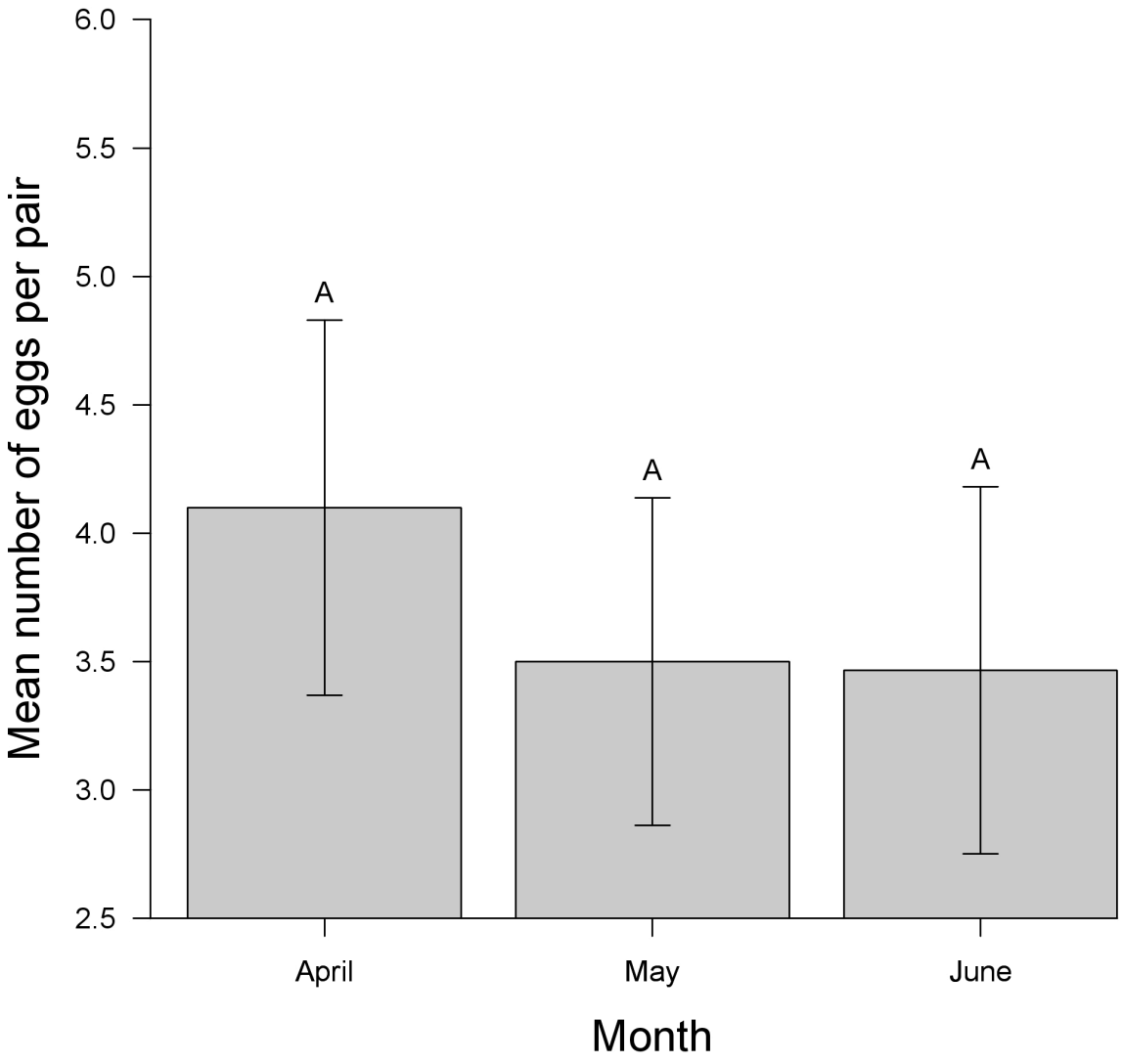

6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
Mean number of eggs per pair
A
A
A
April
May
June
Month

Fig. 3. Histogram of the number of eggs laid by the overwintered adults of *L. maculicollis* on lettuce vein. 30 pairs of beetles collected from a golf course fairway in April 2009 were tested.

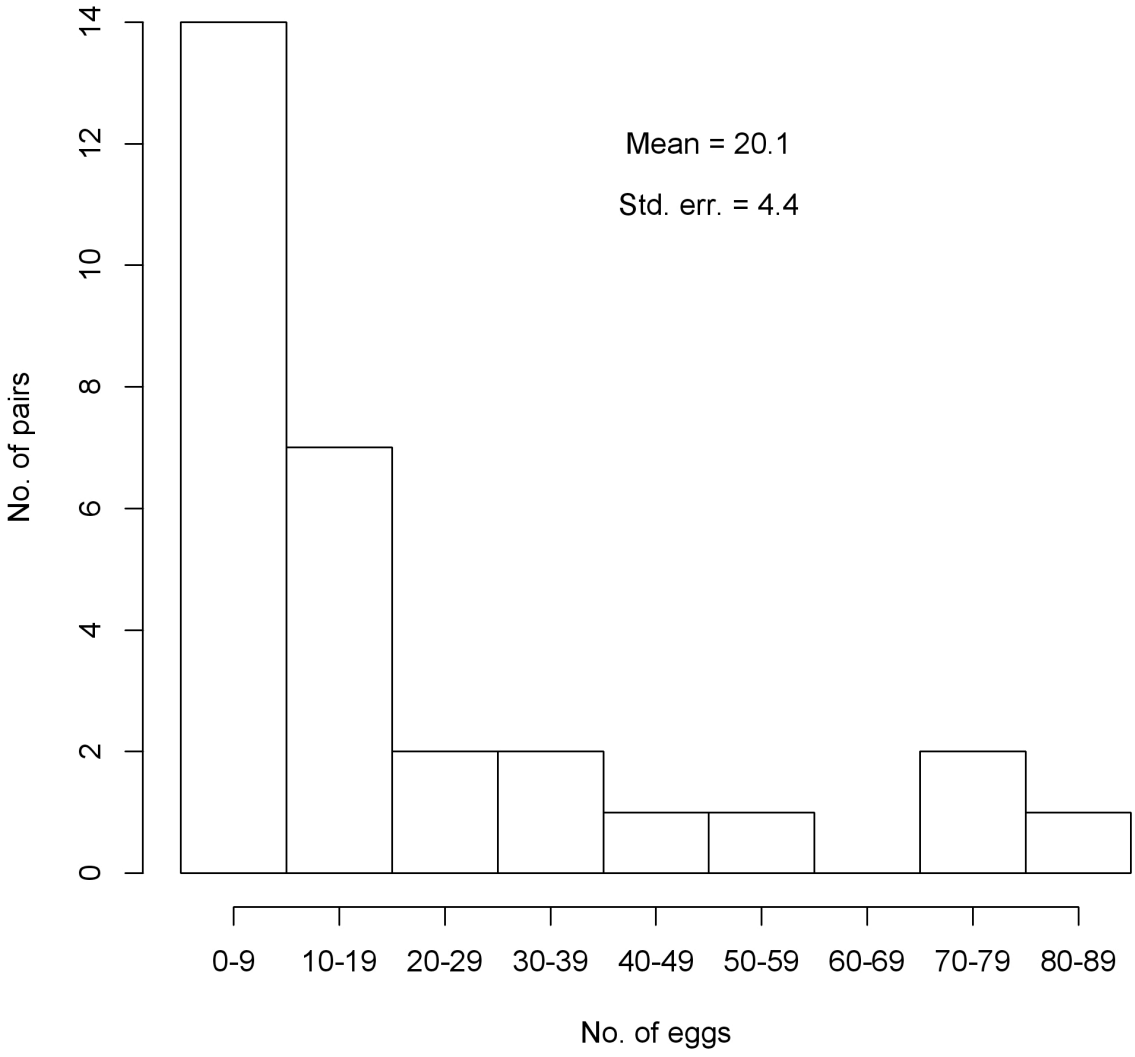

Mean = 20.1
Std. err. = 4.4
No. of pairs
0
2
4
6
8
10
12
14
0-9
10-19
20-29
30-39
40-49
50-59
60-69
70-79
80-89
No. of eggs

Fig. 4. Histogram of the number of eggs laid by *L. maculicollis* adults of the first generation on lettuce vein. 30 paris of the beetles collected from a golf course fairway in July 2009 were tested.

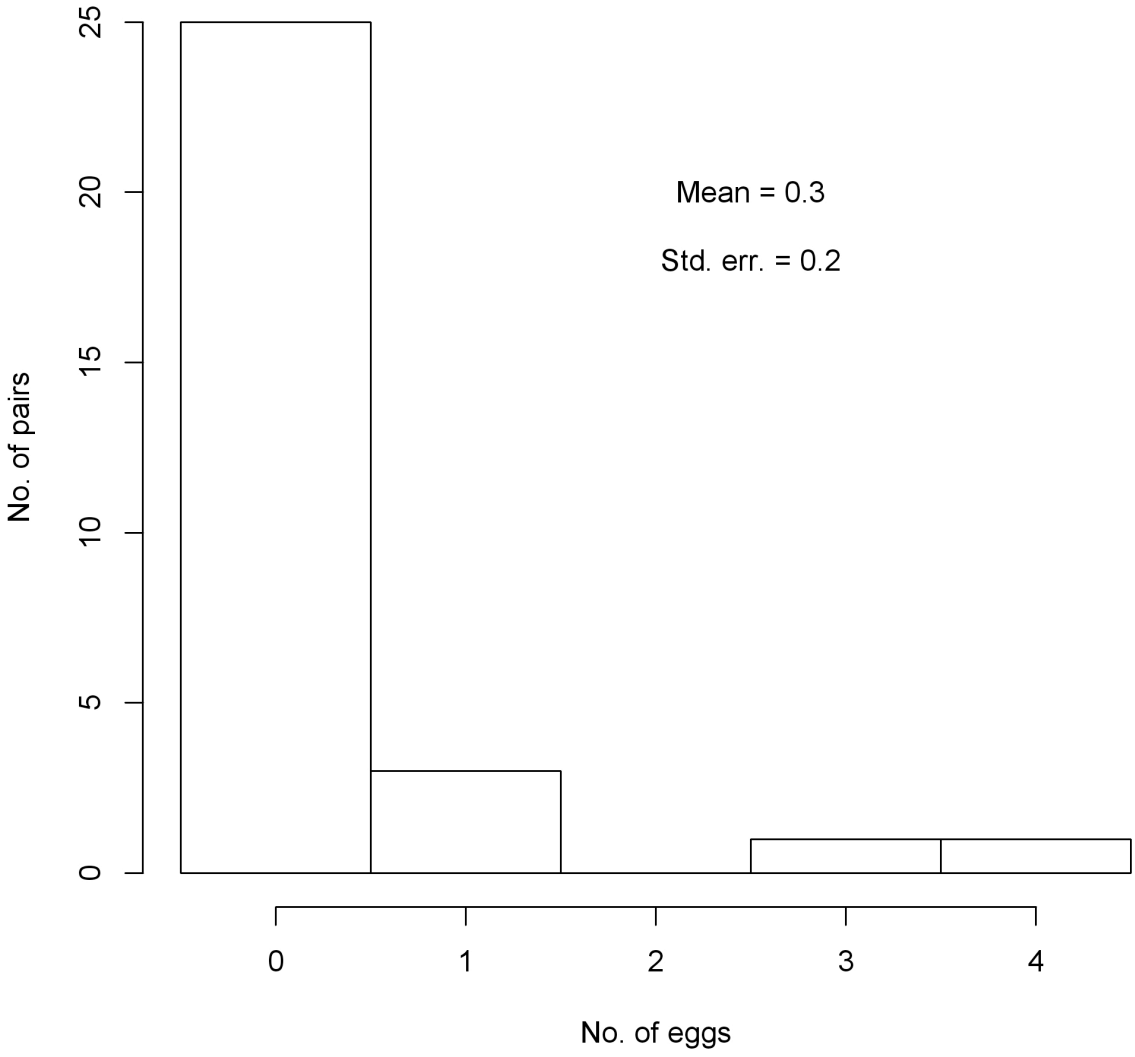

Mean = 0.3
Std. err. = 0.2
No. of pairs
0
5
10
15
20
25
0
1
2
3
4
No. of eggs

Fig. 5. Histogram of developmental times of *L. maculicollis* (males and females combined) reared on lettuce vein from eggs to adults. Eggs were laid in the laboratory by overwintered adults collected from a golf course fairway in April 2009. The Shapiro-Wilk test revealed no significant deviations from the Normal distribution (W = 0.95, $P = 0.34$). The solid line shows a Normal fit.

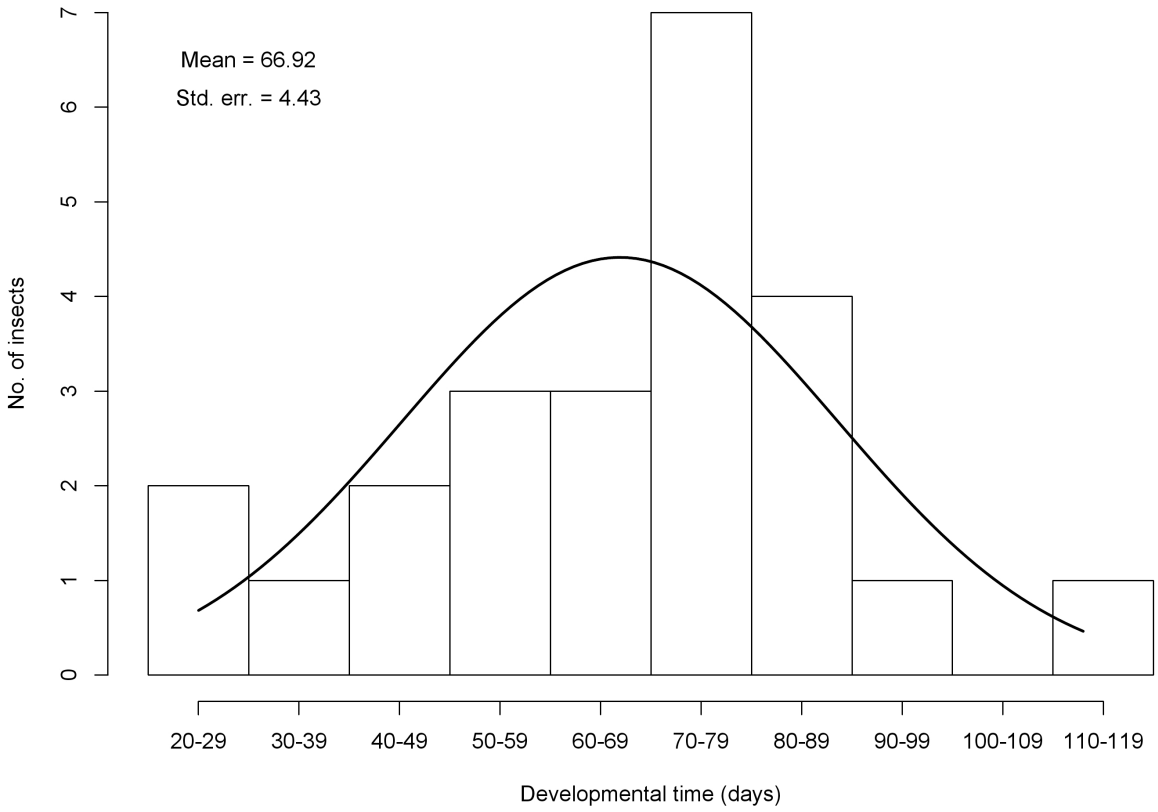
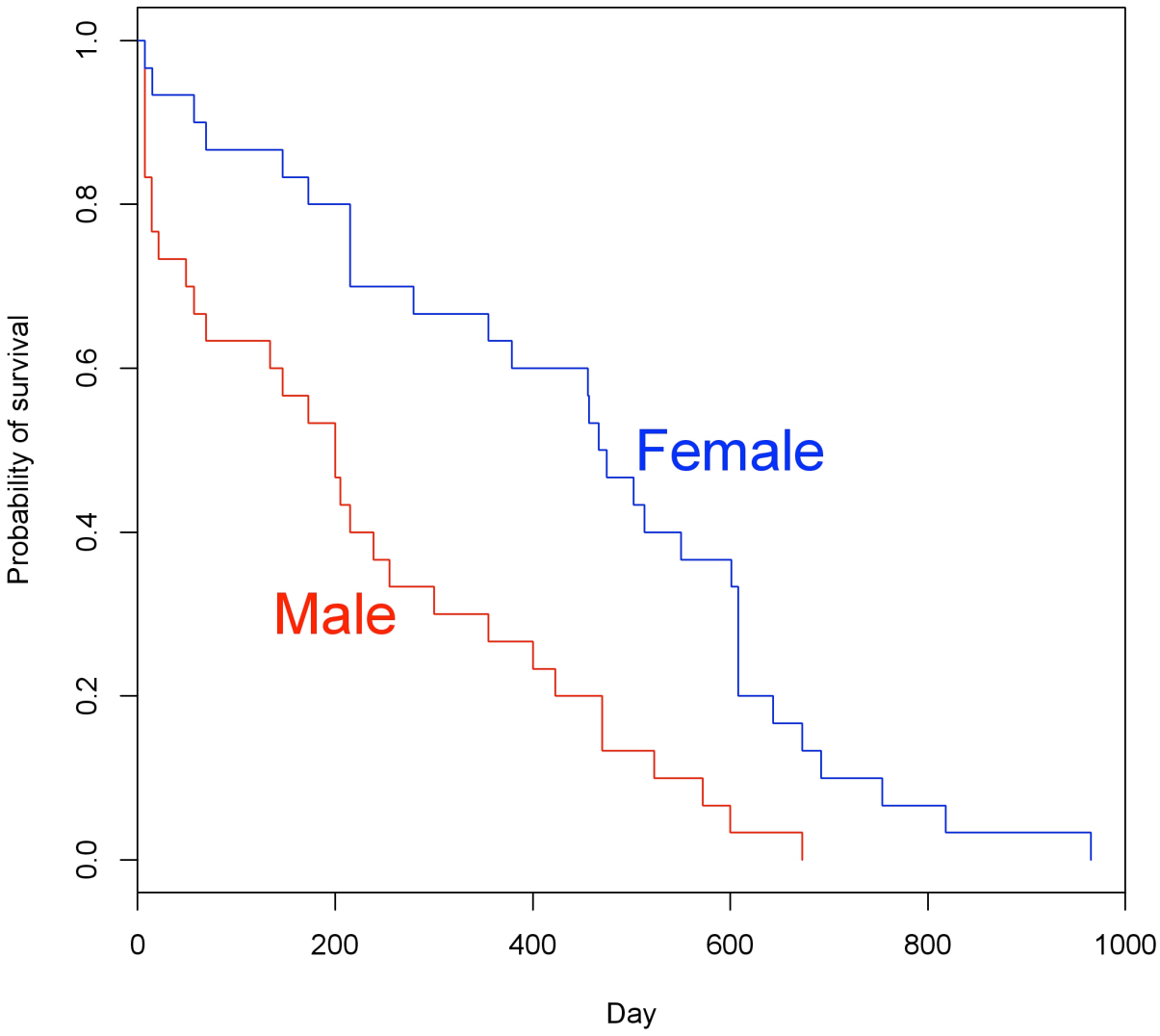

Mean = 66.92
Std. err. = 4.43
No. of insects
0
1
2
3
4
5
6
7
20-29
30-39
40-49
50-59
60-69
70-79
80-89
90-99
100-109
110-119
Developmental time (days)

Fig. 6. Survival curves of adult *L. maculicollis* on artificial diet. 30 pairs of adults collected from a golf course fairway in May 2007 were tested.


Probability of survival
0.0
0.2
0.4
0.6
0.8
1.0
0
200
400
600
800
1000
Day
Female
Male

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